3 1761 07551058 6

The Effect of Certain Agents

on the

Development of Some Moulds

by

atherine olden

K. G. BITTING, M. S. (1869 —

Bacteriologist, Glass Container Association of America

LIBRARY
Pres
NOV 25 1940
UNIVERSITY OF TORONTO

MBa
B

The Effect of Certain Agents

on the

Development of Some Moulds

by

atherine olden

K. G. BITTING, M. S.

Bacteriologist, Glass Container Association of America

Ack to A.W. Bitting [illegible] MD

TX
607
B57

NATIONAL CAPITAL PRESS, INC., WASHINGTON, D. C.

During the years in which the writer has been engaged in food research work, various manufacturers have persistently raised the question of how spices and other condimental agents may be used as preservatives, and it has been taught frequently that these may be used for such purposes. During this same period, many samples of food stuffs were examined which were not sterilized, but which showed peculiar and abnormal growths of the organisms present. This led to a study of the effects of various agents found naturally in, or frequently added, to foods, and for further comparative work, other well-known substances were also tested. The results presented represent a summary of studies covering a period of about fifteen years.

The writer wishes to tender thanks and appreciation to Dr. J. C. Arthur, mycologist, Purdue Experiment Station, and Dr. R. L. Emerson, noted toxicologist, formerly assistant chief, Bureau of Chemistry, U. S. Department of Agriculture.

THE PHYSIOLOGICAL EFFECT OF VARIOUS AGENTS ON THE DEVELOPMENT OF PENICILLIUM EXPANSUM, ALTERNARIA SOLANI, AND OIDIUM LACTIS

INTRODUCTION

It is well known that many chemicals, drugs, and ordinary household substances possess preservative properties to a greater or less degree, depending to some extent upon how they are used. In order to determine the physiological effect of some of these agents upon living matter, a series of experiments was made, using three moulds, which are well known, *Penicillium expansum* Link, *Alternaria solani* (E. & M.) Jones and Grout, and *Oidium lactis* Fresenius. In much of the work of this nature, the tests have been made on either the low organisms, the bacteria, or the higher forms of plants and animals, the results being necessarily confined to the bare facts of retardation or inhibition of development for the former, as they are too minute to note structural changes other than those of form, and for the latter are confined to diagnostic features, as examination of the effect on the tissues is precluded during life. In the present work the nature of the organisms selected permits of minute changes in structure being seen during life as well as the more obvious changes in kind and degree of development. In the preliminary work, a yeast was used in conjunction with the moulds, but the yeast development could not be determined in turbid solutions nor on some solid media, nor could slight changes in the microscopic structure be recorded readily by means of photomicrographs, which were used throughout the work for permanent records and for comparison. As the elimination of this feature of the work precluded close interpretation of the results, the work on the yeast was discontinued.

The records for the experiments are given as to the effect of the different agents used in varying degrees of concentration,usually to the highest point at which growth occurred. Following is a brief description of the moulds grown under conditions which permit normal development.

Penicillium expansum Link.—The Penicillium in developing forms a white, felt-like mass, covering the medium on which it is growing. As development proceeds, the mould changes to bluish green, due to conidial development, then to olive green, finally to a darker, duller color. Thom[1] states that, "The shades of color peculiar to each species under oft repeated conditions are easily recognized and are quite reliable."

Alternaria solani (E. and M.) Jones and Grout.—Alternaria forms a white abundant growth at first, which darkens later, due to the darkening of the hyphal walls and the formation of the brown conidia. During the early stages of development, the mycelium becomes of a greyish color, due to the darkening of the reverse side of the mycelium, but later the whole growth becomes very dark and under some conditions wholly black.

---

[1] Cultural Studies of Species of Penicillium. Bull. 188, B. A. I., Dept. of Agr., 16, 1910.

Oidium lactis Fresenius.—The Oidium differs from the Penicillium and Alternaria in that it remains white throughout its development. During the early stages of growth, it remains submerged in liquid media, then thin white patches of film are formed, which gradually spread and thicken to form a thick, wrinkled scum, that becomes powdery in appearance, as the hyphae divide in forming the conidia.

The Penicillium was isolated from tomato pulp and also from a mild tomato ketchup; the Alternaria from blighted tomatoes; and the Oidium from mature tomatoes allowed to stand until they softened and rotted. The Oidium also develops frequently on pulp, which is allowed to stand after exposure to the air.

Medium.—The medium selected was tomato bouillon as it was noted that the three organisms grew luxuriantly upon this medium, and also upon tomato gelatin, as well as upon such tomato products, as pulp and purée. Before deciding upon the use of this medium, other media tested were the ordinary nutrient bouillon, gelatin, and agar, and diluted fruit juices, pea bouillon, and wort. The tomato bouillon proved most satisfactory as it was slightly acid, gave a seemingly normal development, and could be obtained readily throughout the year. The bouillon was made by adding to a definite volume of cooked, ripe tomatoes an equal volume of water, boiling for half an hour, then filtering. The filtrate is a clear, amber-colored liquid, having an acidity of approximately 0.15 per cent, estimated as acetic acid. In the preparation of the medium in the flasks, calendered paper was tied over the cotton plugs during the sterilization, and after inoculation the paper caps were kept over the plugs to keep them free from dust, and to prevent, to an extent, evaporation.

In the preliminary experiments both moist chambers and flask cultures were used, but the results in the moist chambers were not uniform, neither was the action of the preservative so pronounced as in the flask cultures, due undoubtedly to the small amount of solution which could be used, and the exhaustion of the oxygen in the chamber, and since the latter factor alone produces either an inhibition or an abnormal development, this method of culture was abandoned.

Developmental Features.—The features noted in the development of the organisms were those showing the influence of the agent tested on the germination of the conidia, on vegetative development, and on reproduction, these being manifested in the period required for germination, the extent of growth in a definite period, the form of growth, the formation of conidia, the time required for maturation, as evidenced by the chromatic changes, and any irregularity in form, color, or extent. In addition to these features, microscopic examination was made of the surface and submerged growths to determine the effect on the protoplasm and cell-walls and any irregularities of development. The microscopic examination proved to be much the better method of noting the effects of the agents, for in many cases irregularities were present which were not apparent to the naked eye, and which would pass unnoticed if only the macroscopic features were examined.

In drawing conclusions as to the effect of any substance tested, the visible changes taking place in the protoplasm have been considered in conjunction with the gross developmental features.

Inoculations.—The material used for the inoculations was taken from young cultures grown on the stock medium. The inoculations were made with a platinum needle, having the tip hammered flat and smooth. This was touched to the dry spores in the case of the Penicillium, then tapped on the inside of the flask to dislodge any excess of spores, then touched to the surface of the culture liquid, after which the flask was shaken vigorously to distribute the spores. This method gave a fairly even seeding. With the Alternaria the mycelium had to be used for seeding, the smallest portions possible being cut with the edge of the flattened needle. While the inoculating portion varied in size, the resulting germination gave fairly uniform results. The Oidium, like the Penicillium, permitted even seeding, the needle being touched lightly to the powdery surface.

Temperature.—As the number of cultures was large and, in many cases where germination was retarded, had to be kept under observation for months, they could not be cared for in an ordinary incubating oven, so were placed on shelves in a large case that was protected from light and from changes in temperature, the temperature being approximately 23°C.

Agents.—Solutions of the agents under investigation are given in percentage terms throughout for uniformity, as gram-molecule solutions could not be made of the spices and some of the other substances which were tested. The chemicals used were chemically pure, the spices were tested microscopically for purity, the salt and sugar were the household forms without additional purification, and the drugs were obtained from a reliable source.

Many tests were made in order to determine the inhibiting point of the agent, and when this was approximated, tests were made with solutions varying by slight amounts from one another and in the case of the Alternaria inoculations made from cultures of different ages as this organism showed some irregularities in germination. Many repetitions were made, but only those made in consecutive order are recorded in this paper. The same is true of the photographs, many being held as records which are not shown.

Record of Results.—In recording the results, two sets of tables are used. In the first table are given the number of days required for germination and the macroscopic developmental features of the growths in the flasks, the latter at intervals of twenty-four hours for the first five days. Where the time required for germination exceeded five days, this part of the record is necessarily blank. The second set of tables records the average size of the conidia of the Penicillium after germination (in cases showing extremes in size, two figures are given, the average of the smaller and that of the larger) and the microscopical appearance. The descriptions here are supplemented by the photographs, which should be examined with a hand lens in order to compare advantageously the normal structure with that induced by the various agents. The conidia of the Alternaria and Oidium were not measured. Those of the Alternaria vary very considerably in size ($145–370\mu \times 16–18\mu$)[1] during their development under normal conditions and those of the Oidium vary both in size and shape, so that it would be impossible to differentiate the extent of the variation due to the agent.

---

[1] F. L. Stevens: The fungi which cause plant disease: 623, 1913.

Classification of Agents.—The substances used in the experiments were classified into the following groups. In the grouping the fundamental principle of chemical composition was eliminated in order to group substances which are used for analogous purposes. The last two groups were used for comparison.

1. The oldest and most generally used preservatives: salt, sugar, potassium nitrate.
2. The common spices and aromatics: allspice, celery, cinnamon, cloves, curry,[1] garlic, ginger, mace, mustard, paprika, pepper—black and white—cayenne pepper, sage.
3. Some vegetable acids found in, or which are added to, foods: vinegars—cider, malt, distilled—acetic, butyric, citric, lactic, malic, tannic, and tartaric acids.
4. Some preservatives which have been, and are, used in foods: alcohol, benzoic acid, sodium benzoate, boric acid, borax, creosote, formaldehyde, formic acid, sodium formate, saccharin, salicylic acid, sodium salicylate.
5. Some substances which have been classed as antiseptics, disinfectants, or as having an injurious effect upon living matter: alum, copper sulphate, ferrous sulphate, lead acetate, sodium sulphate, sodium sulphite, stannous oxide, stannous phosphate, stannous protochloride, stannous sulphate, zinc chloride.
6. Three mineral acids—hydrochloric, nitric, and sulphuric; and two common alkalis—potassium and sodium hydroxide.
7. Some very active drugs and some poisons: atropin sulphate, cocain hydrochlorate, morphin sulphate, strychnin nitrate, quinin sulphate, mercuric chloride, carbolic and oxalic acids.

Preservatives.—In the conservation of food the object has been to retain it in as nearly the original condition as possible, and in attaining this object various substances have been used, some of them, like salt, since remote antiquity. Some of them are used also, as the salt is, not only to aid in the preservation, but to enhance flavor. Though the use of preservatives furnishes an easy method of conservation, it is attended with objections, since the effect on the consumer is not properly understood, nor how long the preservative will be effective unless used in excessive amounts.

FIRST GROUP

The members of this group are the oldest and best known food preservatives, salt and sugar particularly having been prepared and used in very ancient times.[2] These have been, and are, used in the flavoring of foods as well as in the drying, pickling, and preserving, both alone and combined. The saltpeter has been used to partially restore the color of meats which has been destroyed in the pickling.

---

[1] Though curry is a mixture of spices, it is used in practically the same way as the spices, and for this reason was included in the tests.

[2] A. Rolet: Les Conserves de Fruits, 1912; H. M. Robinson and C. H. Cribb. The Law and Chemistry of Food and Drugs, 1895; C. H. Gill. British Manufacturing Industries, 1876.

Developmental Features

Agents Kind	Per cent	No. days to germinate	2 days	3 days	4 days	5 days
Penicillium:						
Control....	..	1	Ring, blue spots, many submerged colonies.	Ring ½ inch, older part blue.	Ring ¾ inch, green; surface colonies.	Surface covered, olive.
Salt......	10	2	Many tiny submerged colonies.	Few surface colonies.	Increased.	Colonies curled, blue.
Salt..	15	5	....	....	....	Tiny submerged colonies.
Sugar...	25	2	Thin ring at edge...	Ring ¼ inch wide, edge blue.	Small, surface colonies.	Ring thick, surface nearly covered, green
Sugar...	50	4	....	....	Ring barely perceptible.	Thin ring, green.
Sugar............	66⅔	15	....	....	....	....
Potassium nitrate......	2	1	Ring ¼ inch, blue, small surface colonies.	Nearly covered, blue.	Covered, green; many submerged colonies.	
Potassium nitrate......	10	1	Thin ring, many surface colonies, blue.	Enlarged, green......	Nearly covered, green.	Slightly less than 7 per cent.
Potassium nitrate......	25	2	Few tiny submerged colonies.	Surface nearly covered with tiny transparent colonies.	Thin white layer.	Layer thickened, blue spots.
Potassium nitrate......	30	2	Many tiny surface colonies.	Surface nearly covered with tiny transparent colonies.	Surface covered, colonies united.	Thicker, blue spot.
Alternaria:						
Control..........	.....	2	Many surface and submerged colonies..	Number of submerged colonies increased.	Surface greenish, reverse dark.	Surface covered, reverse and submerged darkened.
Salt.......	5	2	Many tiny submerged colonies.	Few tiny surface col., sub. increased.	Surface greenish, white at edge.	Surface covered, thin, reverse yellowish.
Salt.........	10	3	....	Many tiny submerged colonies.		Slight increase in size.

Developmental Features—Continued

Agents		No. days to germinate	2 days	3 days	4 days	5 days
Kind	Per cent					
Salt	15	17	......	......	......	......
Sugar	25	2	Few submerged colonies.	Number of colonies increased.	......	Liquid full of small colonies, none on surface.
Sugar	50	5	......	......	......	One small submerged colony.
Potassium nitrate	5	2	Many submerged colonies.	Few surface colonies.	......	Surface colonies greenish, curled.
Potassium nitrate	10	2	Many submerged colonies smaller than 5 per cent.	Few surface colonies.	Surface slightly colored.	
Potassium nitrate	20	13	......	......	......	......
Oidium:						
Control	1	1	Surface covered, dry, wrinkled.			
Salt	5	1	Many submerged and surface colonies.	Surface covered, thick dry spots.		
Salt	10	30	......	......	......	......
Sugar	25	1	Surface covered, thin.	Thick dry spots.		
Sugar	50	21	......	......	......	......
Potassium nitrate	5	1	Surface covered, thin	......	Like control.	
Potassium nitrate	10	1	Few surface colonies.	Covered, thin.		
Potassium nitrate	20	13	......	......	......	......

HISTOLOGICAL FEATURES

Agents		Size of germinated conidia in μ	Characteristics of development
Kind	Per cent		
Penicillium			
Control	...	8.5	Hyphae somewhat irregular in outline near germinated conidia, tapering tips; protoplasm homogeneous, many large round vacuoles.
Salt	5	7.6	Hyphae short, distorted, blunt tips; protoplasm homogeneous, no vacuoles. As growth becomes older, development becomes more nearly normal.
Salt	10	7.6	Some hyphae have many septa, irregular branching, many close together, blunt tips or some enlarged, protoplasm apparently normal, many vacuoles.
Salt	15	7.6	Hyphae have many side branches with narrow angles to main hyphae, so that all run in same general direction; protoplasm homogeneous, many vacuoles; fruiting heads normal in shape, but small.
Sugar	50	7.6	Hyphae shrunken, distorted; protoplasm homogeneous, vacuoles appear as pink spots, giving a beaded appearance.
Potassium nitrate	5	7.6	Normal.
Potassium nitrate	10	6.7	Hyphae slightly thinner, otherwise normal.
Potassium nitrate	30	7.6	Hyphae have short stumpy side branches, wall irregular in outline; protoplasm homogeneous, many vacuoles.
Alternaria			
Control	...	...	Hyphae regular appearance; protoplasm finely granular.
Salt	5	...	Hyphae enlarged, thickened appearance, septation increased; protoplasm normal.
Salt	10	...	Hyphae shrunken, rounded, distorted, septation decreased; protoplasm has coagulated appearance.
Salt	15	...	Hyphae ragged in appearance, septation decreased; protoplasm granular.
Sugar	25	...	Normal size, attenuated appearance, septation increased; protoplasm thin, many vacuoles.
Sugar	50	...	Hyphae shrunken, septation increased; protoplasm attenuated, highly vacuolated.
Potassium nitrate	10	...	Hyphae much matted, clean appearance; protoplasm homogeneous, few vacuoles.
Oidium			
Control	...	...	Few hyphae, conidia mostly rectangular, protoplasm finely granular, large vacuoles.
Salt	5	...	No long hyphae, septation hastened, protoplasm finely granular, few small vacuoles.
Salt	10	...	Hyphae and conidia somewhat shrunken, protoplasm homogeneous, large vacuoles.
Sugar	25	...	Septation delayed for 48 hours, protoplasm thin, indistinct, large vacuoles.
Sugar	50	...	Hyphae swollen, irregular conidia distorted, protoplasm disorganized.
Potassium nitrate	5	...	Hyphae septated irregularly, conidia square ends; protoplasm granular, elongated vacuoles.
Potassium nitrate	10	...	Hyphae unseptated; protoplasm homogeneous, no vacuoles.

The effect of the salt was to retard growth and to produce a stunted development, the Oidium being more susceptible to its action than the other two organisms, no development of the Oidium taking place in the 15 per cent solution. The colonies of the other two organisms developed very slowly and with irregular, curled edges. The sugar also retarded and produced stunted development, causing the Oidium to form a hard brownish lump. The Penicillium and Alternaria developed thin surface mycelia, and in the Penicillium particularly there was formed a proportionately larger amount of spores. In making the 66⅔ per cent solution, it was necessary to heat it over the direct flame in order to bring the sugar into solution. When this was effected, it was sterilized for the same length of time as were the others. Growth of the Penicillium in this solution was delayed for so long a time, that the sugar had formed a mass of crystals, on which the growth appeared as tiny grey patches. No development of the other organisms occurred in this strength solution. The salt caused the hyphae to have a smooth, shining appearance, whereas, the sugar caused an excessive vacuolation, the vacuoles being regular and prominent, and with a pinkish tint, so that they gave the appearance of a necklace to the hyphae.

The potassium nitrate was tested in low per cent solutions first, as it was supposed from some of the literature[1] and also its use in meat pickling, that it had strong preservative properties, but beyond a slight retardation, the development was apparently normal for the Penicillium, even in the 25 per cent solution where crystals were separating. In the 30 per cent solution a mass of crystals formed in the bottom of the flask, so that a mount could not be made without them. In this solution, though the development was retarded to a greater extent than in the 25 per cent solution, the surface was covered with a fairly thick mycelium in 4 days, so that beyond retarding growth slightly, the nitrate was not one-half so effective as a preservative as was sodium chloride.

In an article[2] on the use of saltpeter in pickling meats, it is stated that its effectiveness in practice is probably due to its conversion to nitrites or nitric oxid, though usually its effectiveness is attributed to the osmotic activity which it induces.

The effect produced on the organisms by the salt, sugar, and potassium nitrate was merely plasmolysis. So far as could be judged by the appearance under the microscope, and the after effects as evidenced by the use of the cultures for inoculations into ordinary media, no permanent injury resulted. It will be noted that in all the work the agent tested is not alone, but in a balanced nutrient solution, which gives, when used alone, a normal development of the organisms.

See plates 1–7.

SECOND GROUP

The members of this group, comprise some of the spices and aromatic substances of ordinary household use. These were first tested in the form of infusions, made according to the method of the U. S. Pharmacopoeia,[3] and with Penicillium only. With the exception of allspice, cinnamon, cloves, and mustard, the other aromatics had such slight antiseptic value that they were tested by using them direct. The whole substances were ground, 5 grams of each placed in flasks and 10 c.c. of boiling tomato bouillon added. On cooling, they were inoculated with the Penicillium. Included with these were whole black and white mustard seeds, which in household practice are often added to cider, and other home-made beverages in order to preserve them.

---

[1] A. Herzen. Deut. Chem. Ges., Ber. 8:822.

[2] H. Schroeter, Pure products, 1908, 4:199.

[3] U. S. Dispensatory, 19th Ed.:651.

The results from the use of the infusions and the whole ground aromatics are given in the table, the Alternaria and Oidium being used in the infusions having the more pronounced antiseptic properties.

Condiments		No. days to germinate	Developmental Features			
Kind	Per cent		2 days	3 days	4 days	5 days
Penicillium:						
Control		1	Ring, blue spots; many submerged colonies.	Ring one-half inch, older part blue.	Ring three-fourths inch, green; surface colonies.	Surface nearly covered, green.
Allspice, ground	1	2	Tiny surface colonies	Number increased.	Surface nearly covered with small curled colonies.	Covered, blue spot.
Allspice, ground	2	19				
Celery seed	2	1	Surface covered.	Surface blue.	Surface green.	
Celery stalk[1]	2	1	Surface covered.	Blue in center.	Surface blue.	Surface green.
Cinnamon[2]	5	2	Large colonies at edge.	Enlarged, blue centers, many submerged.	Number increased, older ones green.	Surface nearly covered, thick, wrinkled olive.
Cinnamon	10	3		One small colony	Enlarged, center green.	Number increased, oldest one olive.
Cloves[3]	5	2	Thin ring at edge	Ring three-eighths inch, edge blue; many submerged colonies.	Ring one-half inch, edge green.	Ring thick, wrinkled; surface colonies.
Cloves	10	3		Tiny colonies at edge.	No change.	Colonies curled.
Curry	33⅓	2	Colonies dotting surface.	Surface nearly covered, blue spots.	Covered, blue	Surface green.
Ginger	33⅓	1	Surface nearly covered, blue spots.	Surface covered, blue.	Surface green.	
Mace	33⅓	2	Colonies dotting surface.	Surface nearly covered; blue spots.	Surface green.	
Mustard	0.2	7				
Mustard	0.5	14				
Mustard	1	16				

[1] Stalks dried and ground.
[2] Infusion — 100 c.c. contained 0.0194 extractive.
[3] Infusion — 100 c.c. contained 0.0779 extractive.

DEVELOPMENTAL FEATURES—Continued

Condiments		No. days to germinate	2 days	3 days	4 days	5 days
Kind	Per cent					
Mustard, black seed	25	2	Thin interrupted ring.	No change	Increased; surface colonies.	Many surface colonies.
Mustard, white seed	25	2	Thin interrupted ring.	Enlarged, blue spots.	Increased; surface colonies.	Surface colonies enlarged, blue.
Paprika, Hungarian	33⅓	2	Small colonies	Surface one-third covered, blue spots.	Surface covered, blue.	Surface green, overgrown with Mucor.
Paprika, ordinary	33⅓	2	Small colonies	Surface one third covered, blue spots.	Surface covered, blue.	Surface green, overgrown with Mucor.
Pepper, cayenne	33⅓	3		Small colonies	Colonies enlarged	Colonies blue.
Pepper, black	33⅓	1	Surface nearly covered, blue spots.	Surface covered, blue.	Surface green	Overgrown with Mucor.
Pepper, white	33⅓	1	Surface nearly covered.	Surface covered, blue.	Surface green.	
Sage[1]	33⅓	1	Small colonies	Surface nearly covered blue.	Surface covered, green.	
Alternaria:						
Allspice	5	3		Submerged colony		Small surface colony.
Allspice	10	5				Submerged colony.
Cinnamon	10	3		Few small sub. colonies.	Slight increase	One thin surface colony.
Cloves	5	3		Small surface colony.		Slightly enlarged.
Cloves	10	6				
Mustard	1	4			Few submerged col. and at edge.	Increase in size, garlic odor.
Oidium:						
Allspice	5	1	Thin film, many sub. colonies			Slightly wrinkled, thin.
Cinnamon	15	5				Few tiny hard dots at edge.
Cloves	20	4			Tiny dots on surf. and sub.	
Mustard	0.5	2	Many tiny sub. col.		Surface covered, thick	
Mustard	1	4			Few small surf. spots.	Surface covered.

[1] Leaves dried and ground.

Histological Features

Agents		Size of germinated conidia in μ	Characteristics
Kind	Per cent		
Penicillium			
Allspice	1	7.6	Tendency to form fruiting heads from side branches, otherwise normal in appearance.
Cinnamon[1]	10	13.3	Hyphae swollen, few septa in some, more in others and prominent, few side branches, blunt tips, protoplasm finely granular, without cohesion; walls and protoplasm disorganized, break with weight of cover-glass, or when mounted in water.
Cloves[2]	10	13.3	Hyphae swollen, short thick side branches, tips blunt, thicker than older part; protoplasm finely granular, not so badly disorganized as in cinnamon solution.
Mustard	0.5	15.2	A tuft of short, irregular hyphae develop from main branch, the walls have small irregular projections—the undeveloped branches—protoplasm reduced to limiting membrane and threads enclosing large vacuoles. Some long hyphae, many septa, finely granular protoplasm.
Alternaria			
Allspice	2	...	Hyphae somewhat attenuated and irregular.
Cinnamon	10	.	Hyphae ragged, weakened appearance; protoplasm disorganized.
Cloves	10	..	Similar to cinnamon.
Mustard	1	...	Hyphae somewhat enlarged, ragged, septation decreased; protoplasm coarsely granular.
Oidium			
Allspice	5		
Cinnamon	15	...	Hyphae enlarged, hazy appearance; protoplasm reduced.
Cloves	20	...	Hyphae enlarged ,weakened appearance; protoplasm reduced.
Mustard	1	....	Practically normal; conidia more nearly spherical.

The results indicate that curry, ginger, mace, the paprikas—Hungarian and the pungent[3]—the peppers, and sage possess little or no antiseptic value, though they are usually credited with assisting in the preservation of food. The mace used was the Banda, the other two kinds, the Bombay and Papua, were eliminated as they became covered with mould in the laboratory. Garlic was also eliminated, the bulblets when separated and kept in a glass jar, also becoming covered with mould.

Since allspice, cinnamon, and cloves showed the highest antiseptic values, tests were made to determine the amount of the active principle extracted from each one during the process of heating, substituting cassia for cinnamon, since that is the form used generally on account of its greater abundance and lesser cost. Definite amounts were boiled respectively in weak acid solutions for 30 minutes with the following results:

---

[1] Infusion gave in 100 c.c.—0.0194 extractive.

[2] Infusion gave in 100 c.c.—0.0779 extractive.

[3] H. C. Irish. Revision of the Genus Capsicum, 9th Ann. Rep. Missouri Botanical Garden, April, 1898.

Spice	Per cent of oil extracted
Allspice	33.3
Cassia	27.8
Cloves	11.5

There is a general conception that spices are particularly effective as antiseptic agents. This has been reflected many times by manufacturers attempting to use them as preservatives in the preparation of table condiments. Their effect when used for flavoring purposes may be illustrated by taking ketchup as an example. A standard small batch of ketchup contains 50 gallons, weighing approximately 9 pounds per gallon, thus giving 450 pounds or 7,200 ounces. The important spices used in ketchup are allspice, cassia or cinnamon, and cloves. These are often used in the proportion of 6, 8, 7 ounces respectively in a mild ketchup.

If six ounces of allspice be used in the formula, the proportion of the whole spice to the ketchup would be as 1 to 1,200. The active principle is the oil, of which there is about 4 per cent present, making the proportion 0.24 ounce to the batch. But as only 33.3 per cent is extracted, the effective part would be only 0.08 of an ounce or in the proportion of 1 to 90,000.

Overman and Sayer[1] give the antiseptic value of oil of allspice as 1 to 140 and 1 to 180. The antiseptic property of the oil is ascribed to eugenol which is present in the proportion of 65 per cent, thus being present in the batch in the proportion of 1 to 138,461.

If the cinnamon be used in the proportion of 8 ounces to the batch, it would be in the ratio of 1 to 900. The active principle is the oil which is present in the ordinary grade in the proportion of about 1¼ per cent, making the amount only 0.1 ounce to the batch. But as only 27.8 per cent is extracted, there would be present 0.0278 of an ounce or in the proportion of 1 to 258,993.

Cassia contains essentially double the amount of oil that cinnamon does, and is used as a substitute for the latter, therefore the proportion would be 1 to 129,496. Peck[2] gives the antiseptic value of oil of cassia as 1 to 2100. Overman and Sayer give it as low as 1 to 300.

The antiseptic principle in the oil of cassia is cinnamic aldehyde and constitutes about 75 per cent of the whole. The proportion present would be 1 to 345,322 where cinnamon is used and 1 to 171,660 where cassia is used.

Whole cloves when used as in the foregoing formula, 7 ounces to the batch, would be present in the proportion of 1 to 1,029. The oil is the active ingredient which is present in about 18 per cent or in the whole amount there would be 1.26 ounces of which only 11 per cent is extracted, or 0.1386 ounce, making the proportion 1 to 51,948. Peck gives the antiseptic value of oil of cloves as 1 to 1,150, and Overman and Sayer give the value as between 1 to 160 and 1 to 200.

---

[1] C. J. Overman and L. E. Sayer. Merck's Report, 1897, 6: 279.
[2] A. H. Peck. Jour. Amer. Med. Ass., 1889, 32: 6-11.

The active principle in the oil is the eugenol which is present to the extent of 80 per cent making the proportion in the batch equivalent to 1 to 64,935.

The figures indicate that very little of the antiseptic is obtained ordinarily from the spices in foods in which they are cooked for a short period. In estimating the antiseptic value, it has been customary to consider the amount existing in the spice rather than the amount extracted, thus giving them a higher antiseptic value than they possess, though this error naturally is not committed when the estimation is based on experiment.

In the making of ketchup, the proportion of spices will vary as will also the length of time of cooking, but in no case can the amount used be sufficiently great to exercise an appreciable antiseptic effect, which naturally holds true also for other foods. If used in amounts sufficient to have an antiseptic effect, the flavor of the spices would be intolerable.

The spices which are antiseptic in the smaller amounts—allspice, cinnamon, cloves, and mustard—when used in amounts sufficient to produce marked retardation, cause disorganization of both the cell-wall and the protoplasm, the mustard in addition producing peculiar distortions of form, showing that the spices are injurious to the organisms used in the tests.

See Plates 8–13.

THIRD GROUP

This group comprises some organic acids which are normally present in vegetable tissues, and others which are the result of the activity of micro-organisms, and were tested for their effect on the development of the moulds. The moulds are said generally to thrive in acid media, and on acid fruits and other foods, but the statement needs qualification as to the kind of acid.

In testing the acetic acid, both pure acetic acid and vinegars manufactured by the quick process were used. In preliminary experiments, in which slow process vinegars were also used, the action of the vinegars on the development of the Penicillium lacked uniformity when compared with the action of a corresponding percentage of acetic acid. The vinegars were tested for acidity in terms of acetic acid before being used, and were sometimes turbid and of unknown composition. When vinegars of known composition, made by the quick process, and free from extraneous matters were used, the results were uniform and corresponded closely with those of acetic acid of equal strength, so that only the acetic acid was used in the tests on the Alternaria and Oidium. The citric, lactic, and malic acids were also eliminated from the tests on the latter organisms on account of their slight antiseptic action.

Butyric acid acts as a preservative in some substances. It is formed in milk, bread, and other foods, and is made apparent through the rancid flavor and odor. It is the product of organisms which are capable of acting on starch, dextrin, sugar, and even on cellulose. Since it is present in the end fermentation of so many foods, it was inferred that it possessed antiseptic value in that it hindered further fermentative or putrefactive action.

Developmental Features

Agents		No. days to germinate	2 days	3 days	4 days	5 days
Kind	Per cent					
Penicillium:						
Control		1	Thick ring, blue spots, many submerged colonies.	Ring, $\frac{1}{2}$ inch, older part blue.	Ring $\frac{3}{4}$ inch, green; surface colonies.	Surface nearly covered, green.
Acetic acid	0.5	2	Few surface colonies.	Surface one-third covered.	Surface covered, blue.	Surface green.
Acetic acid	1	4	....	....	Few surface colonies, curled.	Slight increase, blue spots.
Cider vinegar	0.5	2	Few surface colonies.	Surface one-third covered.	Surface covered, blue.	Surface green.
Cider vinegar	1	4	....	....	Few surface colonies.	Slight increase.
Malt vinegar	0.5	2	Few surface colonies.	Slight increase.	Surface one-fourth covered, blue.	Surface one-half covered, green.
Malt vinegar	1	4	....	....	Few surface colonies, curled.	Slight increase.
Distilled vinegar	0.5	2	Few surface and few submerged colonies.	Slight increase.	Surface one-fourth covered, blue.	Surface three-fourths covered, green.
Distilled vinegar	1	4	....	....	Few surface colonies, curled.	Slight increase, blue spots.
Butyric acid	0.2	4	....	....	Few tiny surface colonies.	Increase, blue spots.
Citric acid	5	2	Few surface and submerged colonies.	Many surface colonies, blue.	Surface one-half covered, blue.	Surface two-thirds covered, blue.
Citric acid	6	2	Slightly less than the 5 per cent.	Many surface colonies, blue spots.	Surface one-half covered, blue.	Surface two-thirds covered, blue.
Citric acid	10	2	Few tiny surface colonies.	Many small curled surface and tiny submerged colonies.	Increase, blue spots.	Surface covered with separate colonies, blue.
Lactic acid	5	2	Few surface and submerged colonies.	Surface nearly covered.	Blue.	Covered, blue.
Lactic acid	6	2	Less than the 5 per cent.	About one-half the amount of the 5 per cent.	One-half covered, blue.	Two-thirds covered, blue.

DEVELOPMENTAL FEATURES—Continued

Agents		No. days to germinate	2 days	3 days	4 days	5 days
Kind	Per cent					
Lactic acid	10	6				
Malic acid	5	2	Few surface and submerged colonies.	Many surface colonies, blue.	Surface nearly covered, blue.	Surface covered, blue.
Malic acid	6	2	Few surface and submerged colonies.	Many surface colonies, blue.	Surface nearly covered, blue.	Surface covered, blue.
Malic acid	10	2	Few tiny surface colonies.	Many small surface and submerged colonies.	Increase, blue spots	Surface covered with separate colonies, blue.
Tannic acid	3	3		Few tiny surface colonies.	Increased	One-third of surface covered.
Tartaric acid	4	2	Many tiny surface and submerged colonies.	Many curled colonies, blue spots.	Surface one-half covered, blue.	Nearly covered, green.
Alternaria:						
Acetic acid	0.03	3		Submerged colony		Slight increase.
Acetic acid	0.04	4			Submerged colony.	
Butyric acid	0.2	5				Submerged colony.
Tannic acid	0.5	4			Submerged colony.	
Tartaric acid						
Oidium:						
Acetic acid	0.2	1	Thin, film, many submerged colonies.			
Acetic acid	0.5	8				
Butyric acid	0.1	3		Many tiny submerged colonies.		Surface nearly covered.
Tannic acid	0.5	4			Submerged colony.	
Tartaric acid						

HISTOLOGICAL FEATURES

Agents		Size of germ. conidia in μ	Characteristics
Kind	Per cent		
Penicillium			
Acetic acid.......	0.5	15.2	Hyphae swollen, tips blunt, protoplasm coarsely granular, filling cells in some cases, others have large vacuoles, both walls and protoplasm disorganized, break easily.
Acetic acid.......	1	19.0	More swollen than the ½ per cent, protoplasm coarser, fills cells to bursting, breaks more easily.
Cider vinegar.....	0.5	15.2	Like ½ per cent acetic acid.
Cider vinegar.....	1	19.0	Like 1 per cent acetic acid.
Malt vinegar......	0.5	15.2	Like ½ per cent acetic acid.
Malt vinegar......	1	19.0	Like 1 per cent acetic acid.
Distilled vinegar...	0.5	15.2	Like ½ per cent acetic acid.
Distilled vinegar...	1	19.0	Like 1 per cent acetic acid.
Butyric acid......	0.2	19.9	Hyphae swollen, wide tips, protoplasm coarsely granular, no vacuoles; protoplasm and walls disorganized. Many hyphae broken in mounting.
Citric acid........	10	11.4	Hyphae enlarged close to germinated spore, some with many septa, tapering, thin; protoplasm homogeneous, many small vacuoles; in others large vacuoles, protoplasm thin appearance.
Lactic acid.......	10	15.2 49.4	Conidia swollen, some with coarsely granular, yellow protoplasm, others have clear protoplasm, much vacuolated; hyphae swollen, distorted, protoplasm has characteristics of germinated conidia. Some cells of the hyphae are swollen, pear-shaped and round, as are also some of the end cells.
Malic acid........	10	13.3	Hyphae swollen near germinated spore, septa form short cells, from this tapering, thin; protoplasm homogeneous, small vacuoles in some, in others large and elongated, walls break easily.
Tannic acid.......	3	11.4	Hyphae vary in size, some large, empty, septa close together; others attenuated, septa normal, protoplasm homogeneous.
Tartaric acid......	4	11.4	Hyphae two kinds, large with coarse protoplasm, small with homogeneous protoplasm and many vacuoles. Small hyphae have many short side branches, having wide angles to main branch.
Alternaria			
Acetic acid.......	0.04	....	Hyphae normal size, walls and protoplasm disorganized.
Butyric acid......	0.2	....	Hyphae have ragged appearance, septation increased; protoplasm coarsely granular.
Oidium			
Acetic acid.......	0.5	....	Hyphae shrunken, septation irregular; protoplasm coarsely granular.
Butyric acid......	0.1	....	Hyphae swollen, hazy appearance, septation reduced; protoplasm thin, granular.

The acetic acid had a decided effect in retarding and inhibiting the growth of the organisms, the Penicillium being the least susceptible of the three to its action, growth taking place in the 1 per cent solution. The 0.5 per cent delayed the germination, but after a certain amount of development had taken place, there was no further effect, as judged by the macroscopic appearance. The 1 per cent delayed germination for 3 days, and after that the development was slow, and the surface colonies curled.

Though the growths in the quick process vinegars showed slight differences, they were practically uniform with the acetic acid, in time of germination and in their action on the structure of the mould, producing marked swelling and disorganization. To estimate the effect adequately, the size of the germinated conidia should be noted and the appearance as shown by the photographs.

With the Oidium, the acetic acid inhibited growth in all solutions having the acid in excess of 0.5 per cent, and in this solution germination was delayed for 8 days.

The Alternaria was much more sensitive than the other two, no development occurring in solutions containing more than 0.04 per cent. In cultures containing 0.02, 0.03, and 0.04 per cent respectively the development showed a decided progressive retardation, for even these minute differences.

The effect on all the organisms was to cause a disorganization of both the cell-wall and the protoplasm, the wall disintegrating and the protoplasm flowing out in the form of coarse granules without any apparent cohesion.

The butyric acid has decided antiseptic value, the 0.2 per cent solution causing a retardation in the Penicillium equivalent to that of the 1 per cent acetic acid. The effect on the other organisms was also strong, no development of the Oidium occurring in solutions containing more than 0.1 per cent, though the histological appearance was not so marked as in the Penicillium.

The citric, lactic, and malic acids gave somewhat similar results in the solutions of 4, 5, and 6 per cent, but when the amount was increased to 10 per cent, the lactic acid was more strongly antiseptic; development started in two days in the citric and malic solutions, but was delayed 6 days in the lactic, and after that developed only tiny white curled colonies on the surface and a few submerged ones, whereas, the others formed fruiting heads in 5 days and made a fairly abundant growth. A peculiar feature of the growth in the 10 per cent lactic acid solution was the development of "giant" cells in the hyphae and from these peculiar branch formations. (See photographs Nos. 1–3, Plate 17.)

The tannic and tartaric acids are stronger antiseptically than the three preceding acids, the 3 and 4 per cent solutions respectively producing an effect equal to that of the 10 per cent of either citric or malic acid.

The citric, lactic, and malic acids were not tested with the Alternaria and Oidium, as they possessed such weak antiseptic properties.

See Plates 14–19.

FOURTH GROUP

The members of this group are the antiseptics which have been used more or less by manufacturers of condimental preparations, by brewers, also in the conservation of milk and raw meats, and which under disguising titles, singly or in combination, have been used in the home preparation of fruits and vegetables. While legally forbidden to manufacturers in most states, they have entered somewhat freely into domestic practice, and some of them into food in interstate commerce.

DEVELOPMENTAL FEATURES

Agents		No. days to germinate	2 days	3 days	4 days	5 days
Kind	Per cent					
Penicillium:						
Alcohol	7	3	.....	Few tiny edge colonies.	Slight increase	Few surface colonies.
Alcohol	9.0	7[1]	.....	.....	.....	.....
Benzoic acid	0.1	6	.....	.....	.....	.....
Sodium benzoate	0.2	8	.....	.....	.....	.....
Boric acid	0.2	3	.....	Few submerged colonies.	Few surface colonies	Thin surface layer.
Boric acid	0.5	7	.....	.....	.....	.....
Borax	0.2	3	.....	Thin ring, few submerged colonies.	Slight increase	Ring and colonies wrinkled, blue spots.
Borax	0.5	6		.....	.....	.....
Creosote	0.2	2	Thin ring, few surface colonies.	Colonies blue, few submerged colonies.	.....	No apparent increase.
Creosote	0.4	4	.....	.....	Few tiny curled colonies.	Interrupted ring of small colonies.
Formaldehyde	0.04	1	Few small colonies ...	Thin interrupted ring.	Many surface colonies, blue.	Increased.
Formaldehyde	0.1	7	.....	.....	.....	.....
Formic acid	0.2	2	Thin ring, few submerged colonies.	Ring enlarged, blue...	Surface colonies, blue, many submerged.	Surface nearly covered, green.
Formic acid	0.5	5	.....	.....	.....	Few edge and submerged colonies.
Sodium formate	1.0	2	Thin interrupted ring, few submerged colonies.	Many surface colonies.	Enlarged slightly, blue.	Surface nearly covered, green spots.
Saccharin	0.5	2	Tiny edge and submerged colonies.	Interrupted ring, surface colonies curled.	.....	One-third of surface covered, blue.
Saccharin	1.0	22	.....	.....	.....	.....

---

[1] Conidia enlarged as seen in photomicrograph, no mycelium developed.

Developmental Features—Continued

Agents		No. days to germinate	2 days	3 days	4 days	5 days
Kind	Per cent					
Salicylic acid	0.2	4			Few tiny surface and submerged colonies.	Slight increase.
Sodium salicylate	0.5	5				Two tiny curled colonies.
Alternaria:						
Alcohol	5.0	2	Many tiny submerged colonies.			Colonies larger.
Benzoic acid	0.01	2	Few tiny submerged colonies.			Slight increase.
Sodium benzoate	0.01	2	Few tiny submerged colonies.			Slight increase.
Boric acid	0.5	4			Many submerged colonies.	
Borax	0.5	5				Submerged colony.
Creosote	0.01	1	Tiny submerged colonies.			Slightly less than control.
Creosote	0.02	6				
Formaldehyde	0.02	1	One tiny submerged colony.			Few small submerged colonies.
Formic acid	0.5	7				
Sodium formate	0.5	3		Submerged colony		Slight increase.
Saccharin	0.2	3		Many submerged colonies.		Increase in size.
Salicylic acid	0.02	2	Few small submerged colonies.			Slightly increased.
Sodium salicylate	0.05	5				Tiny submerged colony.
Oidium:						
Alcohol	5.0	2	Many surface and submerged colonies.	Surface covered.		

DEVELOPMENTAL FEATURES—Continued

Agents		No. days to germinate	2 days	3 days	4 days	5 days
Kind	Per cent					
Benzoic acid	0.02	5				Many surface and submerged colonies.
Sodium benzoate	0.03	3		Many small submerged colonies.		Surface covered, white, thick.
Boric acid	0.2	3		Many small submerged colonies.		Few surface colonies.
Borax	0.5	7				
Creosote	0.05	3		One tiny submerged colony.		Many small submerged colonies.
Formaldehyde	0.1	4			Few submerged colonies.	Slight increase.
Formic acid	0.2	7				
Sodium formate	0.5	3		Many submerged colonies.	Thin film	Thick, white.
Saccharin	0.3	2	Many submerged colonies.		Surface covered.	
Salicylic acid	0.05	3		Few submerged colonies.		Increased.
Sodium salicylate	0.05	1	Thin film, many submerged colonies.	Thick, white.		

Histological Features

Agents		Size of germ. conidia in μ	Characteristics of development
Kind	Per cent		
Penicillium			
Alcohol	5.0	15.2	Hyphae swollen, distorted, walls tough; protoplasm clear.
Benzoic acid	0.1	49.4	Hyphae larger than in benzoate, more easily broken, distorted.
Sodium benzoate	0.2	15.2 38.0	Hyphae and conidia swollen and distorted, no uniformity in formation of septa, some hyphae have few, others many; protoplasm coarsely granular, filling tubes; walls break readily.
Boric acid	0.2	15.2 19.0	Hyphae swollen, short thick side branches, blunt ends, protoplasm finely or coarsely granular.
Creosote	0.04	9.5	Hyphae nearly as wide as germinated spore, many septa; protoplasm homogeneous, many fine vacuoles, many short side branches develop into fruiting heads, having few branches.
Formaldehyde	0.1	7.6	Hyphae attenuated, septa distinct; protoplasm appears solid, shrunken from walls. Fruiting heads have stringy branches, conidia have refractive greenish content, shrunken.
Formic acid	0.2	14.0	Hyphae swollen, coarsely granular, blunt tips, many broken, or, normal size, finely granular, many vacuoles.
Formic acid	0.5	11.4 41.8	Hyphae swollen, coarsely granular, yellow, distorted, badly disorganized, break easily. Nearly all germinated conidia broken.
Sodium formate	0.2	13.3	Hyphae swollen, short side branches, blunt tips; protoplasm coarsely granular, disorganized; or, hyphae normal size, protoplasm finely granular; some cells empty.
Sodium formate	0.5	13.6	Hyphae swollen, short undeveloped side branches, blunt tips; protoplasm coarsely granular, disorganized, walls break easily.
Saccharin	0.2	13.3	Hyphae enlarged, some much swollen slight distortion; protoplasm clear, homogeneous; conidiophores thick, stunted.
Salicylic acid	0.2	15.2	Hyphae and conidia swollen, some of the conidia much elongated, few septa, tips blunt; protoplasm coarsely granular, disorganized, also the walls.
Sodium salicylate	0.2	9.5	Hyphae as wide as germinated conidia, few septa, granular protoplasm.
Alternaria			
Alcohol	5.0	....	Hyphae swollen septation reduced; protoplasm coarse, disorganized.
Benzoic acid	0.02	....	Hyphae enlarged, protoplasm and walls disorganized.
Sodium benzoate	0.03	....	Hyphae enlarged, protoplasm and walls disorganized.
Boric acid	0.2	....	Hyphae enlarged, protoplasm weakened, has clear appearance.
Borax	0.5	....	Hyphae much weakened, protoplasm reduced.
Creosote	0.05	....	Hyphae slightly enlarged, protoplasm and walls disorganized, septation reduced.
Formaldehyde	0.1	....	Some hyphae swollen, others shrunken, hazy appearance; protoplasm badly disorganized.

HISTOLOGICAL FEATURES—Continued

Agents		Size of germ. conidia in μ	Characteristics of development
Kind	Per cent		
Formic acid	0.2		Hyphae enlarged, protoplasm and walls disorganized.
Sodium formate	0.5		Hyphae enlarged, protoplasm and walls disorganized.
Saccharin	0.3		Normal size, protoplasm non-hyaline, swollen appearance.
Salicylic acid	0.05		Normal size, protoplasm non-hyaline, swollen appearance.
Sodium salicylate	0.05		Some hyphae swollen, protoplasm disorganized.
Oidium			
Alcohol	5.0		Hyphae swollen, septation reduced; protoplasm coarse, disorganized.
Benzoic acid	0.02		Hyphae slightly swollen, walls and protoplasm weakened, break easily. Some hyphae entirely disorganized, protoplasm reduced, coarsely granular.
Sodium benzoate	0.03		Hyphae slightly swollen, walls and protoplasm weakened, break easily.
Boric acid	0.2		Hyphae enlarged, protoplasm weakened, has clear appearance.
Borax	0.5		Hyphae much weakened, protoplasm reduced.
Creosote	0.05		Size normal, but disorganized appearance, septation reduced.
Formaldehyde	0.1		Some hyphae swollen, others shrunken, hazy appearance; protoplasm badly disorganized.
Formic acid	0.2		Hyphae enlarged, protoplasm and walls disorganized.
Sodium formate	0.5		Hyphae enlarged, protoplasm and walls disorganized.
Saccharin	0.3		Normal size, protoplasm non-hyaline, swollen appearance.
Salicylic acid	0.05		Normal size, protoplasm non-hyaline, swollen appearance.
Sodium salicylate	0.05		Some hyphae swollen, protoplasm disorganized.

The results of the tests with these preservatives show the acid to be stronger in all cases than the corresponding salt,[1] and also that the benzoic acid was the strongest antiseptic in its action on the three moulds. In testing the preservatives in this group, the ordinary beef gelatin and tomato gelatin were used as well as the tomato bouillon, but the gelatins were found unsatisfactory on account of irregularity in development. The gelatins permitted only a surface growth, which limited the absorbent action of the hyphae to the part in contact only, whereas, in the solution the entire surface of the hyphae acts as an absorbent. The sodium benzoate, as it was the most generally used preservative, was tested under a greater variation of conditions than the others, and presented many peculiar features. In some cases, the development might show a greater retardation, but less effect on structure, than in others where the development seemed more nearly to approach the normal in extent, but on microscopic examination a more marked effect on structure could be seen. In some tests where the smaller amounts of preservative were used, the disorganization of both the protoplasm and the cell-walls in the early development was as great as with the larger amounts, while the later development might be almost normal, as if the entire effect were concentrated in the first growth, or some colonies might show a marked effect and others little or no effect, both developing simultaneously.

---

[1] E. L. Trouessart: Antiseptic Therapeutics, Vol. 1, 1893. Trans. by E. P. Hurd.

The Alternaria and Oidium were much more sensitive to the action of both the benzoic acid and the sodium benzoate, the solutions in which growth occurred being only about one-tenth the strength of those in which the Penicillium was able to develop. Similar selective effect to that in the Penicillium was also apparent in these organisms, some hyphae showing the disorganization as in the Penicillium, while others appeared normal.

Disorganization of both protoplasm and cell-walls resulted in varying degrees from the action of the preservatives in this group. The disorganization was manifested in swelling and distortion, the protoplasm becoming coarsely granular and without cohesion, there being no perceptible distinction between the limiting membrane and plasma, also in varying degrees of yellowing or browning, and in rupture from the mere handling in the transference to the slide, or the weight of the cover-glass, or the change in osmotic action due to the mounting in a drop of water instead of the medium in which grown. The protoplasm, when colored, had a rusty, dirty appearance, not the clear color which is produced when stained. When the wall ruptured, the protoplasm flowed out, the coarse granules spreading in all directions, showing total lack of cohesive force. Buchanan[1] states that salicylic acid has the peculiar property of macerating flesh, so that the hands are attacked, when it is used as an antiseptic, and on account of this property it constitutes one of the ingredients of "corn salves." The other members of this group which behave practically alike in their effect on the moulds, apparently do not possess this property, though butyric acid which is also similar in its effect on the moulds has been used by Loeb[2] to destroy the superficial cortical layer of the unfertilized egg before development can take place.

The Alternaria and Oidium could not be tested in so close a series as the Penicillium as their growth was inhibited more easily; in the one case a fairly good development was obtained with a small amount of the preservative, but a slight increase above this amount completely inhibited development. Very minute amounts of the preservatives were sufficient to produce marked effects, the effects being similar to those obtained on the Penicillium. *See Plates* 20–33.

FIFTH GROUP

In this group are substances which have been classed as antiseptics, disinfectants, or as having an injurious effect on living matter. Alum and copper sulphate have been used in the preparation of certain foods, and the other salts listed have sometimes entered foods from the vessels used in preparation or from the containers, and are said by some writers to be poisonous.[3]

For the tin compounds to be used in the tests the stannous series was selected as these are the ones to which poisonous properties are attributed, though in the government regulation[4] cognizance is not taken of the form in which the tin occurs; the basis for the regulation appears to be the result of chemical analyses, including both the stannic and stannous series.

---

[1] C. M. Buchanan: Antisepsis and Antiseptics, 1895.

[2] J. Loeb: Popular Science Monthly, 80 (1), 1912.

[3] J. W. Holland: Medical Chemistry and Toxicology, 1905; H. Leffmann and W. Beam. Select Methods in Food Analysis, 1905; K. P. McElroy and W. D. Bigelow, Dept. of Agr., Bul. 13, part 8, 1893.

[4] Food Inspection Decision, No. 126.

DEVELOPMENTAL FEATURES

Agents		No. days to germinate	2 days	3 days	4 days	5 days
Kind	Per cent					
Penicillium:						
Alum	0.5	2	Few tiny surface colonies.	Slight increase	Colonies.	
Copper sulphate	1.0	7				
Ferrous sulphate	0.5	2	Many sub. few surf. colonies.	Center of colonies blue.		Colonies green.
Lead acetate	0.05	4			Many small hard submerged colonies.	
Sodium sulphate	2.0	3		Small submerged colonies.		Slight increase in size and number.
Sodium sulphite	0.2	5				Thin, ring, few submerged colonies.
Sodium sulphite	0.5	15				
Stannous oxide	5.0	9				
Stannous phosphate	2.0	10				
Stannous protochloride	0.02	3		Small submerged colonies.		Colonies increased in size, hard and compact.
Stannous sulphate	0.02	3		Small submerged colonies.		Slight increase in size and number.
Zinc chloride	0.2	3		Tiny surface colonies.	Slight increase	Colonies curled, blue spots.
Alternaria:						
Alum	0.5	5				Tiny compact submerged colonies.
Copper sulphate	0.1	2	Many submerged colonies.			Slightly enlarged.
Copper sulphate	0.5	34				
Ferrous sulphate	0.2	2	Few submerged colonies.			Slightly enlarged.
Lead acetate	0.2	3		Few submerged colonies.		No apparent increase.

Developmental Features—Continued

Agents		No. days to germinate	2 days	3 days	4 days	5 days
Kind	Percent					
Sodium sulphate	0.5	2	One colony at edge	...	...	Colony colored, fruiting.
Sodium sulphate	1.0	3	...	One submerged colony	...	No apparent change.
Sodium sulphite	0.2	8	...	...	...	...
Stannous oxide	5.0	3	...	Small submerged colony.	...	Slightly enlarged.
Stannous phosphate	1.0	2	Few small surface colonies.	...	Colonies darkened.	
Stannous protochloride	0.1	4	...	...	Few submerged colonies.	
Stannous sulphate	0.1	11	...	...	...	...
Zinc chloride	0.2	8	...	...	...	...
Zinc chloride	0.5	10	...	...	...	...
Oidium:						
Alum	1.0	2	Many tiny submerged colonies.	...	Many white dry surface colonies.	
Copper sulphate	0.1	1	Surface covered, dirty appearance.			
Ferrous sulphate	1.0	3	...	Tiny white dots on surface.	...	Slight increase.
Lead acetate	1.0	23	...	...	...	...
Sodium sulphate	2.0	1	Many tiny submerged.	Surface covered white.		
Sodium sulphite	1.0	1	Many surface and submerged colonies.			
Stannous oxide	5.0	1	Patches of film	Surface covered.		
Stannous phosphate	1.0	1	Patches of film	Surface covered.		
Stannous protochloride	0.05	1	Many submerged colonies.	Surface covered, thin film.		
Stannous sulphate	0.1	1	Patches of film	Surface covered, white		
Zinc Chloride	0.2	1	Hard white dots on surface, many submerged.	Surface covered.		
Zinc Chloride	0.5	10	...	...	...	...

HISTOLOGICAL FEATURES

Agents		Size of germinated conidia in μ	Characteristics of development
Kind	Per cent		
Penicillium			
Alum	1.0	11.4 19.0	Large round cells are formed from germinated conidia from which are developed attenuated hyphae. Fruiting heads developed close to germinated spores, some of which are thin and small.
Copper sulphate	0.2	11.4	Hyphae enlarged, slightly distorted; protoplasm yellow, finely granular, has dirty appearance.
Ferrous sulphate	0.05	8.5	Hyphae normal size, many small fruiting heads formed.
Lead acetate	0.05	8.5	Protoplasm coarse disorganized; some strongly vacuolated.
Sodium sulphate	2.0	8.5	Some hyphae enlarged, strongly vacuolated.
Sodium sulphite	0.2	11.4	Hyphae enlarged, few septa; protoplasm coarsely granular.
Stannous oxide	5.0	8.5	Hyphae attenuated, protoplasm coarsely granular.
Stannous phosphate	2.0	11.4	Hyphae badly disorganized, coarse protoplasm.
Stannous protochloride	0.02	11.4	Some hyphae enlarged, disorganized; some shrunken with disorganized protoplasm.
Stannous sulphate	0.02	11.4	Many hyphae swollen, ends break with weight of cover glass; protoplasm coarse, some strongly vacuolated.
Zinc chloride	0.2	4.0 11.4	Two forms of hyphae, one set large, regular, smooth outline, coarsely granular protoplasm; other set small, distorted, protoplasm homogeneous. Fruiting heads small, branches in various directions.
Alternaria			
Alum	0.5	....	Hyphae irregular, ragged appearance, septation increased; protoplasm coarsely granular.
Copper sulphate	0.1	....	Hyphae slightly enlarged, walls and protoplasm disorganized.
Ferrous sulphate	0.2	....	Some hyphae shrunken, septation increased; protoplasm coarsely granular.
Lead acetate	0.2	....	Hyphae empty, septation increased.
Sodium sulphate	1.0	....	Hyphae disorganized, protoplasm coarsely granular.
Sodium sulphite	0.2	....	Hyphae enlarged, protoplasm coarsely granular.
Stannous oxide	5.0	....	Hyphae normal size; protoplasm attenuated, coarsely granular.
Stannous phosphate.	1.0	....	Hyphae enlarged, protoplasm coarsely granular.
Stannous protochloride.	0.2	....	Hyphae normal size; protoplasm attenuated, coarsely granular.
Stannous sulphate	0.1	....	Hyphae disorganized, enlarged.
Zinc chloride	0.5	....	Hyphae enlarged, distorted, break easily; protoplasm coarsely granular escaping from hyphae.
Oidium			
Alum	1.0	....	Hyphae shrunken, septation reduced; protoplasm stringy.
Copper sulphate	0.5	....	Some hyphae enlarged, irregular; protoplasm coarsely granular; others attenuated, disorganized prominent septa.
Ferrous sulphate	1.0	....	Hyphae distorted, attenuated, protoplasm coarse.
Lead acetate	0.5	....	Some hyphae shrunken, irregular; protoplasm attenuated, many vacuoles.

HISTOLOGICAL FEATURES—Continued

Agents		Size of germinated conidia in μ	Characteristics of development
Kind	Per cent		
Sodium sulphate...	2.0		Swollen, disorganized, homogeneous protoplasm.
Sodium sulphite...	3.0		Slightly enlarged.
Stannous oxide....	5.0		Hyphae have ragged appearance; protoplasm reduced, coarse.
Stannous phosphate.	3.0		Hyphae enlarged, protoplasm coarsely granular.
Stannous protochloride.	0.05		Hyphae attenuated, indistinct, septation reduced; protoplasm coarse.
Stannous sulphate.	0.05		Hyphae normal size, ragged appearance; protoplasm coarse.
Zinc chloride......	0.5		Hyphae enlarged, irregular; protoplasm coarsely granular

The Alternaria was the most susceptible of the organisms to the action of the alum, both in checking development and histologic effect. Similar results, though with different amounts of the alum were produced in all, evidenced in an impoverished-appearing protoplasm, the hyphae appearing somewhat clear and more attenuated as the amount of the alum was increased to the inhibiting point, and the protoplasm more reduced.

The copper sulphate in the lesser amounts produced enlargement and disorganization, the protoplasm being rather coarsely granular and lacking cohesion. In the larger amounts, germination was retarded, the hyphae were stunted and, in some cases, misshapen. Clark,[1] using molar solutions, found that N/128 (0.1 per cent) inhibited growth in P. glaucum, and that N/1 was required to kill the mould. Pfeffer[2] states that P. glaucum is still able to grow in 21 per cent. They undoubtedly worked with different strains of what was known as P. glaucum. Alsberg and Black[3] found that species of Penicillium so closely related that until recently they were not distinguished by morphologists, differ quite markedly in their metabolism. The writer found that in the development of the Penicillium used in the tests the organism was more sensitive to slight changes in environment, as shown morphologically, as well as being more resistive, as shown in development when transferred to normal media, when the inoculations into the test media were made from cultures which had been kept in vigorous condition through transfers made fairly regularly and at somewhat short intervals.

---

[1] J. F. Clark: On the toxic effect of deleterious agents on the germination and development of certain filamentous fungi. Bot. Gaz., 28: 289, 375, 1899.

[2] W. Pfeffer: Physiology of Plants, Vol. II: 60, 1906.

[3] C. L. Alsberg and O. F. Black: Contributions to the study of maize deteriorations. Bul. 270, B. P. I., Dept. of Agr.

The ferrous sulphate and zinc chloride in the largest amounts produced enlargement and disorganization, the latter manifested by the rupture of the walls and the free flowing out of the coarsely granular protoplasm. Pfeffer states that P. glaucum is able to grow in solutions of ferric sulphate up to 8 per cent, which would indicate that the ferric salt was less poisonous. The lead acetate produced an impoverished development and enlargement. The sodium sulphate and sodium sulphite produced swelling and disorganization, with coarsely granular protoplasm. The stannous compounds when in amounts close to the inhibiting point produced impoverishment. In the lesser amounts there was no apparent effect on the hyphae. The Penicillium was more susceptible to their action than were the other organisms, which was contrary to the results with the other agents, as the Penicillium was, in general, the most resistive. The Alternaria and Oidium grew freely in the 5 per cent stannous oxide, and as that was so much larger an amount than would ever be present in a food, no larger amounts were tested. These organisms also developed freely and without retardation in the 1 per cent stannous phosphate solutions. The stannous protochloride and stannous sulphate which are more or less hydrolyzed by water[1] were the strongest in checking growth. In their work on food adulterants, McElroy and Bigelow[2] state that "of the possible metallic contaminations, that caused by tin is, next to that of lead, probably the worst." They state further that, "Tin poisoning resulting from the use of canned foods is not often recorded, although it is probable that minor disturbances to health frequently occur from this cause." They do not state what basis they have for arriving at this conclusion, but quote the work of others[3] in which tin compounds have caused the death of a guinea pig, frogs, rabbits, and dogs.

SIXTH GROUP

The mineral acids—hydrochloric, nitric, and sulphuric—are corrosive in their action, and when concentrated destroy organic matter. They were tested in these experiments in order to compare their action on the structure of the moulds with that of the organic acids.

The alkalis are also local corrosives, the corrosive action being due to their property of dissolving protein, saponifying fat, and extracting water from the tissues. Since the moulds usually develop normally in acid solutions, the alkalis were used to test their adaptability in readjusting the metabolic processes to an evidently antagonistic environment, so as to obtain as radical changes as possible in structure for comparison with the changes induced by the preservatives.

---

[1] Alexander Smith: Introduction to Inorganic Chemistry: 693, 1910.
[2] Loc. cit.; 1040-2, 1164-6.
[3] O. Hehner: Analyst, 1880, 5, 218; Chem. Centrbl., 1883, 810.

DEVELOPMENTAL FEATURES

Agents		No. days to germinate	2 days	3 days	4 days	5 days
Kind	Per cent					
Penicillium:						
Hydrochloric acid	0.2	2	Many surface colonies.	Blue spots	Surface two-thirds, covered, blue.	Covered, green.
Hydrochloric acid	0.5	24				
Nitric acid	0.2	3		Many surface colonies.	Blue spots	Surface two-thirds covered, blue.
Nitric acid	0.5	20				
Sulphuric acid	0.1	2	Small surface colonies.	Surface nearly covered, blue.	Covered, green.	
Sulphuric acid	0.2	20				
Potassium hydrate	0.2	2	Few surface colonies.	Many surface colonies, blue spots.	Surface nearly covered, blue.	Few submerged colonies.
Potassium hydrate	0.5	9				
Sodium hydrate	0.2	2	Few surface colonies.	Increased	Largest colonies, blue.	Many colonies, older ones green.
Sodium hydrate	0.5	10				
Alternaria:						
Hydrochloric acid	0.2	2	Many small submerged colonies.			Enlarged.
Hydrochloric acid	0.5	14				
Nitric acid	0.2	5				Few tiny hard colonies on bottom.
Sulphuric acid	0.1	5				Tiny hard dots on bottom of flask.
Potassium hydrate	0.5	3		Few small submerged colonies.		
Sodium hydrate	0.5	4			Few small submerged colonies.	
Oidium:						
Hydrochloric acid	0.5	5				Tiny brown dots on bottom.
Nitric acid	0.5	1	Patches of film		Covered.	
Sulphuric acid	0.1	2	Few surface, many submerged colonies.	White, dry, hard dots on surface.		
Potassium hydrate	0.5	1	Many surface colonies			
Sodium hydrate	0.5	3		Few surface colonies.	Enlarged wet-looking.	Film brown, ugly.

Sp. gr.: HCl, 1.2; HNO_3, 1.42; H_2SO_4, 1.84.

HISTOLOGICAL FEATURES

Agents		Size of germinated conidia in μ	Characteristics of development
Kind	Per cent		
Penicillium			
Hydrochloric acid....	0.5	7.6 15.2	Hyphae distorted, very irregular, thinning and swelling at intervals in some, branching irregular, ends attenuated; protoplasm yellow, finely granular, no vacuoles, has disorganized appearance.
Nitric acid...........	0.5	7.6	Hyphae short, many septa, protoplasm shrunken, forming solid mass separated from walls.
Sulphuric acid.......	0.1	11.4	Hyphae large, regular, many septa, others small, distorted, many branches, extending in various directions; fruiting heads irregular, small, few branches at various angles; protoplasm granular in large hyphae, homogeneous in small ones. Some hyphae develop large, necklace-like cells close to germinated spore.
Potassium hydrate....	0.5	5.7	Hyphae have blunt tips and finely granulated protoplasm, disorganized appearance, break easily.
Sodium hydrate......	0.2	7.6	Hyphae make short curves, clear; protoplasm appears normal, walls and protoplasm break easily.
Alternaria			
Hydrochloric acid....	0.5	....	Hyphae distorted, septation increased; protoplasm transparent.
Nitric acid...........	0.2	....	Hyphae distorted, knotted, septation increased; protoplasm attenuated.
Sulphuric acid.......	0.1	....	Hyphae badly distorted, knotted; protoplasm has coagulated appearance.
Potassium hydrate....	0.5	....	Submerged hyphae enlarged, surface ones attenuated; protoplasm reduced, strongly vacuolated, some coarse.
Sodium hydrate......	0.2	....	Submerged hyphae enlarged; protoplasm thin, transparent.
Oidium			
Hydrochloric acid....	0.5	....	Hyphae closely matted, irregular, reduced in size; protoplasm coagulated in appearance.
Nitric acid...........	0.2	....	Normal appearance.
Sulphuric acid.......	0.1	....	Hyphae closely matted, distorted, septation reduced.
Potassium hydrate....	0.5	....	Hyphae swollen, twisted, septation; protoplasm badly disorganized.
Sodium hydrate......	0.5	....	Hyphae normal size; protoplasm attenuated.

The 0.5 per cent solution of hydrochloric acid had a peculiar effect on the development of the Penicillium. Twenty-four days were required for germination, and after that the development was slow, consisting of small, brown, submerged colonies and some wet looking lumps on the surface of the solution, which bore no resemblance to the ordinary surface colonies. The surface lumps increased gradually in size for forty-six days, then dropped to the bottom of the liquid, after which a few normal appearing colonies developed on the surface. In the 0.2 per cent nitric acid solution, the growth was retarded, but appeared normal. In the 0.5 per cent solution some of the conidia formed short hyphae, others became merely swollen, and all had a disorganized appearance. In the 0.1 per cent sulphuric acid solution the development appeared normal, but the microscope showed the hyphae to be swollen, and to have a ragged disorganized appearance. In the 0.2 per cent solution, a few small colonies, consisting of attenuated hyphae were developed in 20 days, but no further development was apparent.

The Alternaria formed matted, distorted, knobby hyphae, the 0.5 hydrochloric, the 0.2 nitric, and the 0.1 sulphuric acids giving practically the same results, with the exception that the hyphae in the sulphuric acid solution were more shrunken.

The acids had the same general effect on the Oidium as on the two other moulds, causing a gnarled appearance in the hyphae, with an appearance of coagulation of the protoplasm.

The sodium hydroxid was stronger in its action than the potassium hydroxid. The Penicillium developed in a practically normal manner in the 0.2 per cent solution, but in the 0.5 per cent solution development proceeded slowly in the potassium hydroxid solution, forming a few surface colonies which required 18 days to become green; in the sodium hydroxid solution a few tiny colonies were developed in 10 days, which was the extent of the development. In both solutions the hyphae were attenuated and clear, and broke down easily. The effect of the 0.2 per cent solution of sodium hydroxid was about equal to that of the 0.5 per cent potassium hydroxid, judging from the microscopic appearance. This was also true of the Alternaria, the 0.2 per cent sodium hydroxid and the 0.5 per cent potassium hydroxid producing an impoverished and strongly vacuolated protoplasm and weakened cell walls.

For the Oidium a similar effect to that of the Alternaria was produced by 0.5 per cent solutions respectively of both alkalis.

See Plates 46–50.

SEVENTH GROUP

In this group are some alkaloids and poisons. The alkaloids were atropin sulphate, cocain hydrochlorate, morphin sulphate, strychnin nitrate, and quinin sulphate. Mercuric chlorid, carbolic and oxalic acids were used for comparison with these. The first four alkaloids were obtained in the form of tablets, the atropin and strychnin containing 0.01 (0.00065 gram) of a grain, the cocain and morphin 0.25 (0.01625 gram) of a grain.

Agents		No. days to germinate	Developmental Features			
Kind	Per cent		2 days	3 days	4 days	5 days
Penicillium:						
Atropin sulphate	0.0065	2	Thin ring of colonies.	Ring thicker, blue spots.	Slight increase	Surface colonies blue.
Cocain hydrochlorate	0.1625	2	Thin ring of colonies.	Ring thicker, blue spots.	Slight increase	Surface colonies blue
Morphin sulphate	0.1625	2	Thin ring of colonies.	Ring thicker	Ring blue, few surface colonies.	Ring green, colonies blue.
Strychnin nitrate	0.0065	2	Thin ring of colonies.	Ring thicker, blue spots, many surface colonies.	Surface colonies blue.	Surface nearly covered, green.
Quinin sulphate	0.5	2	Many small surface colonies.	Colonies enlarged, rounded.	Surface one-half covered, blue.	Surface nearly covered.
Mercuric chlorid	0.01	2	Thin ring, few surface colonies.	Increased, blue	Ring and surface colonies, green.	Covered, thin, smooth layer.
Carbolic acid	0.1	5				Many small submerged colonies.
Oxalic acid	1.0	9				
Alternaria:						
Mercuric chloride	0.01	2	Few tiny submerged colonies.			Slightly enlarged.
Carbolic acid	0.05	5				Small submerged colony.
Oxalic acid	0.5	7				
Oidium:						
Mercuric chloride	0.02	1	Surface covered, many submerged colonies.			
Carbolic acid	0.1	1	Many small edge and submerged colonies.		Surface covered with yellow waxy colonies.	
Oxalic acid	0.5	1	Many surface and submerged colonies		Enlarged, curled surface colonies.	

Histological Features

Agents		Size of germinated conidia in μ	Characteristics of development
Kind	Per cent		
Penicillium			
Atropin sulphate	0.0065	4.6 6.7	Hyphae thin, clear, curved, protoplasm apparently normal; others larger with coarsely granular protoplasm, some parts collapsed.
Cocain hydrochlorate.	0.1625	8.5	Parts of hyphae seemingly normal, others strongly vacuolated with coarse, granular protoplasm and adjoining these, some empty with walls breaking into fragments.
Morphin sulphate	0.1625	11.4	Some hyphae large with few shining granules, others empty, broken. Some thin, tapering, many side branches, turned in various directions, protoplasm homogeneous.
Strychnin nitrate	0.0065	11.4	Hyphae of two kinds, larger have coarse granular protoplasm, many disorganized; smaller hyphae have many short irregular branches, extending in all directions, seemingly without system, and homogeneous protoplasm. Fruiting heads small, irregular, short branches spreading at various angles.
Quinin sulphate	0.5	7.6	Hyphae have many septa, protoplasm homogeneous, with few vacuoles; in others coarsely granular protoplasm. Fruiting heads irregular, branches at wide angles to each other.
Mercuric chlorid	0.01	7.6	Hyphae regular and uniform, septa close together few side branches, tips fine, tapering; protoplasm homogeneous, many vacuoles.
Carbolic acid	0.1	11.4 34.2	Hyphae short, many septa, short side branches; protoplasm homogeneous clear. Germinated spores vary, some filled with coarsely granular protoplasm, others have yellow shrunken mass.
Oxalic acid	1	6.7	Hyphae form many side branches, some distorted; protoplasm in some hyphae coarsely granular in others finely granular. Some conidia distorted, undeveloped.
Alternaria			
Mercuric chloride	0.01	....	Some hyphae shrunken, ragged, septation increased; protoplasm reduced.
Carbolic acid	0.05	....	Some hyphae shrunken, others swollen, all have protoplasm coarsely granular and apparently coagulated.
Oxalic acid	0.5	....	Hyphae form many side branches, some distorted; protoplasm in some hyphae coarsely granular in others finely granular. Some conidia distorted, undeveloped.
Oidium			
Mercuric chloride	0.02	.....	Hyphae attenuated; protoplasm reduced.
Carbolic acid	0.1	.....	Hyphae attenuated; break easily; protoplasm reduced, some conidia enlarged.
Oxalic acid	0.5	....	Hyphae swollen, irregular, septation reduced; protoplasm coagulated, walls disorganized, protoplasm flowing out.

In these tests the results indicated that the amounts contained in the tablets, which are intended as ordinary doses, were stimulative to development, the maximum amount of growth in the atropin, cocain, and morphin solutions resulting when two of the tablets were used, and in the strychnin with three tablets. When 10 of the tablets were used, a slight retardation resulted, but as this amount is much higher than is ever prescribed, except under extraordinary circumstances, no larger amounts were tested, nor were they tested with the Alternaria and Oidium. The filler in the tablets was lactose, which in the small amounts used was negligible. The tests with quinin showed a slight retardation when 0.5 per cent was used. With the mercuric chlorid 0.02 per cent was inhibitive, though 0.01 exerted only a slight retardation. This was true also of the growth of the Alternaria. The Oidium, however, was able to develop in the 0.02 per cent solution.

The microscopic examination showed the starting of disorganization, manifested by the coarsely granular protoplasm and broken walls in the atropin, cocain, strychnin, and quinin growths. The growth in the morphin showed a granular precipitation and destruction of plasticity, whereas, that in the mercuric chlorid was seemingly normal with the exception of the increased number of septa. The mercuric chlorid caused attenuation and reduction of protoplasm in the Alternaria and Oidium. The carbolic acid caused disorganization of protoplasm in all three organisms, as did also the oxalic acid.

Clark[1] found strychnin to be practically non-toxic to P. glaucum, and undoubtedly this conclusion would be drawn if the macroscopic features alone were considered, as in the 1-100 and 1-50 grain solutions the development was heavier than in the control.

See Plates 51–55.

MIXTURE OF AGENTS.

In the previous experiments on the effect of the various agents, the action of each has been considered singly; in the following experiments, mixtures were used of some of the substances which produced in some cases similar, in other cases, dissimilar effects. These mixtures of substances are in no way comparable to the balanced solutions of Osterhout,[2] the components of which were toxic when used separately, but which were nontoxic when used together, but it should be noted that in all the work the agent under test was added to the tomato bouillon, which contains the salts necessary for a balanced nutrient solution, and which alone gave normal development. In making the mixture-solutions, the practically retarding point of each was used as a basis and a proportionate part of each taken. When two substances were used in the mixture, one-half of the retarding percentage of each was taken; when three were used, one-third of the retarding percentage of each.

In the table are outlined the mixtures used, the amount of each substance, the time required for germination and the characteristics of development. These experiments were conducted on the Penicillium only as that organism was, in general, more resistive to the action of the agents and more satisfactory in permitting interpretation of results.

---

[1] Bot. Gaz., Nov., 1899.

[2] Bot. Gaz., 1909, 47: 148-149.

DEVELOPMENTAL FEATURES OF THE PENICILLIUM

Agents		No. days to germinate	2 days	3 days	4 days	5 days
Kind	Per cent					
Control		1	Ring, blue spots; many surface and submerged colonies.	Nearly covered, blue	Covered, green.	
Salt	7.5	1	Thin ring, few surface many small submerged colonies.	Ring one-eighth inch, edge blue; surface colonies curled.	Ring one-fourth inch, edge green; surface colonies, blue centers	Nearly covered, green.
Sugar	32.5	2	Surface covered with thin colonies.	Blue edge	Green, smooth surface.	
Acetic acid	0.75	1	Like control, but heavier.	Covered, blue edge[1]	Blue, wrinkled	Green.
Sodium benzoate	0.1	4	......	......	Few tiny surface colonies.	Slight increase, blue spot.
Salt—sugar	7.5 32.5	3	......	Many tiny submerged colonies.	Surface nearly covered with tiny colonies.	Covered, close film.
Salt—acetic acid	7.5 0.75	2	Few small surface and submerged colonies.	Surface colonies increased, curled.	Surface one-half covered, blue spots.	Covered, blue.
Salt—benzoate	7.5 0.1	......	......	......	......	......
Sugar—acetic acid	32.5 0.75	2	Surface covered with thin colonies.	Covered[1]	Blue, smooth	Green.
Sugar—sodium benzoate	32.5 0.1	8	......	......	......	......
Acetic acid—sodium benzoate	0.75 0.1	9[2]	......	......	......	......

[1] Heavier growth than control.
[2] Spores swollen, no further development.

Developmental Features of the Penicillium—Continued

Agents		No. days to germinate	2 days	3 days	4 days	5 days
Kind	Per cent					
Sugar—salt—acetic acid—sodium benzoate.	16.25 3.75 0.375 0.05	3		Two tiny surface colonies.	Number increased	Slight increase in size.
Salt—sugar—acetic acid—sodium benzoate.	2.5 $10.83\frac{1}{3}$ 0.25 $0.03\frac{1}{3}$	4			Few small surface colonies.	Many surface colonies, older ones blue.
Sugar—sodium benzoate—salt.	16.25 0.05 3.75	4			Few tiny surface colonies.	Slight increase.
Sugar—salt—acetic acid.	16.25 3.75 0.375	2	Few surface, many small submerged colonies.	Increased	Surface nearly covered, blue spots.	Covered, blue.
Salt—acetic acid—sodium benzoate.	3.75 0.375 0.05	5				Few tiny surface colonies.

The results of the growths in the mixtures indicate that with two exceptions, though theoretically as strong as the single substance, they are not so effective. In the exceptions, the acetic acid and sodium benzoate, which are similar in their action in causing both enlargement and disorganization of the protoplasm and cell wall, the joint effect was to cause the inoculated conidia to become swollen, and a few of them to form promycelia in nine days, but any further development was prevented. In the salt and sodium benzoate solution, no change was apparent in the conidia, yet the substances differ materially in effect when used alone. The hyphae in the sugar and salt solution were clear, but had an impoverished appearance, and the protoplasm was highly vacuolated; those in the sugar and acetic acid solution were both swollen and much vacuolated, a result showing the effect of both substances. Many of the hyphae in the solution of sugar and sodium benzoate were swollen and disorganized to an even greater extent than resulted from the effect of 0.1 per cent of sodium benzoate used alone, but there were present also masses of mycelial threads in which the effect of the sugar predominated, the hyphae being small, containing hyaline granules, and having impoverished fruiting heads which developed close to the germinated spore. Similar effects were produced in the growths in the solutions of sugar, salt, and acetic acid, and of sugar, salt, and sodium benzoate. In the latter solution some of the hyphae were swollen and disorganized, the effect of the sodium benzoate, others were stunted and had prematurely formed, impoverished fruiting heads. The sugar was more energetic than the salt in producing its typical features. The growth in the solution of salt, acetic acid, and sodium benzoate was strongly characterized by swelling and disorganization. The work on smaller fractional parts is not included in the table.

See Plates 56 57.

GERMINATION TESTS

In order to determine the effect upon the vitality of the Penicillium[1] of continued contact with the agents, cultures were made from the original growths in the solutions containing the agents, to tubes of the normal tomato bouillon. The original cultures, after the earlier observations were completed, had been kept at approximately 18° C., and in a comparatively dark place. When possible, inoculations were made from the spore layer of these cultures, but as no development had occurred in some of them, a platinum loop was used for these, so as to obtain, if possible, some of the spores which had been used in the original inoculations, and thus subjected to the influence of the respective agents. As the original cultures were made at intervals, covering a period of four years, they were necessarily of different ages, so that in consequence no definite periods of time for the action of the agents could be used in the germination tests. Reference to the factors in the following table will make this clear. In making the observations on the tests, note was made of the time required for germination, for the formation of conidia, indicated by the blue color, and for the maturing of the conidia, indicated by the change of the blue color to green;

---

[1] The germination tests are reported on the Penicillium only; those on the other moulds will be included in articles to follow.

in addition to these, note was made of any irregularity in development, a permanent record of this being made by means of photomicrographs as in the preceding work. The tabulated results contain the observations cited, and in addition, the age of the original culture from which the inoculation was made. A young, vigorous culture, in conjunction with an old control culture, were used in determining the deviations from the normal which could be ascribed to the effect of the agent.

RESULTS OF GERMINATION TESTS

Development of first generation in the normal solution. Inoculation from cultures grown for varying periods of time in solutions of the respective agents.

Cultures from which inoculations were made. Development in normal solution.

Agents		Number of days in contact with agent	No. of days to		
Kind	Per cent		germinate	form conidia	mature
Control		14	1	2	3
Control		176	2	3	4
Control		183	2	3	4
Control		210	2	3	5
Control		380	2	4	5
Control		423	3	4	6
Control		590	3	6	8
Salt	5	423			
Salt	5	590			
Salt	10	7	2	3	4
Salt	10	106	2	5	6
Salt	10	590			
Salt	15	7	2	4	6
Salt	15	106	3	4	6
Sugar	25	423	2	3	4
Sugar	25	590	2	3	5
Sugar	50	423	4	5	7
Sugar	50	590			
Sugar	66⅔	423	2	3	6
Sugar	66⅔	590	4	8	9
Potassium nitrate	3	28	2	3	5
Potassium nitrate	5	26	2	3	5
Potassium nitrate	7	14	2	3	4
Potassium nitrate	7	113	3	4	6
Potassium nitrate	10	14	2	3	4
Potassium nitrate	10	113	2	4	6
Potassium nitrate	25	7	2	3	5
Potassium nitrate	25	106	2	5	6
Potassium nitrate	30	89	3	6	7
Potassium nitrate	30	169			
Allspice	1	91	3	5	7
Allspice	1	161	2	3	5
Allspice	2	91	2	4	6
Allspice	2	161	5	7	9
Allspice seed	5	91	3	9	11
Allspice seed	5	161			
Allspice seed	10	91	3	8	9

RESULTS OF GERMINATION TESTS—Continued

Cultures from which inoculations were made. Development in normal solution.

Agents		Number of days in contact with agent	No. of days to		
Kind	Per cent		germinate	form conidia	mature
Allspice seed	10	161	...	...	...
Cinnamon (infusion)	10.0	423	3	4	5
Cinnamon (infusion)	10.0	590	3	5	7
Cloves (infusion)	10.0	423	...	...	...
Cloves (infusion)	10.0	590	...	...	...
Mustard, ground	0.2	14	2	3	4
Mustard, ground	0.2	113	2	4	5
Mustard, ground	0.2	183	2	3	5
Mustard, ground	0.5	8	4	9	10
Mustard, ground	0.5	14	5	11	12
Mustard, ground	0.5	113	3	4	7
Mustard, ground	1.0	12	3	4	7
Mustard, ground	2.0	12	7	8	9
Mustard, ground	2.0	118	2	3	5
Mustard, ground	2.0	188	2	3	5
Mustard seed, black	1.0	8	2	3	4
Mustard seed, black	2.0	8	2	3	4
Mustard seed, black	2.0	107	2	5	6
Mustard seed, black	2.0	177	2	3	5
Mustard seed, black	25.0	7	2	3	4
Mustard seed, black	25.0	107	...	...	...
Mustard seed, black	25.0	177	...	...	...
Mustard seed, white	1.0	8	2	3	4
Mustard seed, white	2.0	8	2	3	4
Mustard seed, white	2.0	107	2	4	6
Mustard seed, white	2.0	177	3	4	6
Mustard seed, white	25.0	7	2	3	4
Mustard seed, white	25.0	107	3	5	7
Mustard seed, white	25.0	177	...	...	...
Acetic acid	0.5	210	2	3	6
Acetic acid	0.5	380	2	3	6
Acetic acid	0.5	423	2	4	6
Acetic acid	0.5	590	3	5	7
Acetic acid	1.0	210	2	3	4
Acetic acid	1.0	380	2	3	5
Acetic acid	1.0	423	2	4	6
Acetic acid	1.0	590	...	...	...
Acetic acid	1.5	210	...	...	...
Acetic acid	1.5	380	...	...	...
Acetic acid	2.0	210	...	...	...
Acetic acid	2.0	380	...	...	...
Cider vinegar	0.5	210	2	3	6
Cider vinegar	0.5	380	3	4	6
Cider vinegar	1.0	210	2	2	3
Cider vinegar	1.0	380	2	3	5
Cider vinegar	1.5	210	...	...	...
Cider vinegar	1.5	380	...	...	...
Cider vinegar	2.0	210	...	...	...
Cider vinegar	2.0	380	...	...	...
Malt vinegar	0.5	210	3	4	6
Malt vinegar	0.5	380	2	3	6

RESULTS OF GERMINATION TESTS—Continued

*Cultures from which inoculations were made.			Development in normal solution.		
Agents		Number of days in contact with agent	No. of days to		
Kind	Per cent		germinate	form conidia	mature
Malt vinegar	1.0	210	3	4	6
Malt vinegar	1.0	380	3	5	6
Malt vinegar	1.5	210	....	....	....
Malt vinegar	1.5	380	....	....	....
Malt vinegar	2.0	210	....	....	....
Malt vinegar	2.0	380	....	....	....
Distilled vinegar	0.5	210	2	3	4
Distilled vinegar	0.5	380	2	3	6
Distilled vinegar	1.0	210	2	4	6
Distilled vinegar	1.0	380	3	5	6
Distilled vinegar	1.5	210	....	....	....
Distilled vinegar	1.5	380	....	....	....
Distilled vinegar	2.0	210	....	....	....
Distilled vinegar	2.0	380	....	....	....
Butyric acid	0.1	34	2	2	4
Butyric acid	0.1	132	3	5	7
Butyric acid	0.1	202	2	4	6
Butyric acid	0.2	39	2	2	4
Butyric acid	0.2	138	3	5	8
Butyric acid	0.2	208	2	4	6
Butyric acid	0.5	32	....	....	....
Citric acid	4.0	25	2	3	4
Citric acid	4.0	124	3	5	10
Citric acid	5.0	14	2	3	4
Citric acid	5.0	113	2	5	6
Citric acid	6.0	14	2	3	4
Citric acid	6.0	113	2	4	6
Lactic acid	4.0	25	2	3	4
Lactic acid	4.0	124	2	4	6
Lactic acid	5.0	14	2	2	3
Lactic acid	5.0	113	2	4	6
Lactic acid	6.0	14	2	2	3
Lactic acid	6.0	113	2	4	6
Malic acid	4.0	25	2	3	4
Malic acid	4.0	124	3	7	10
Malic acid	5.0	14	2	2	4
Malic acid	5.0	113	2	5	7
Malic acid	6.0	14	2	3	4
Malic acid	6.0	113	2	4	6
Tartaric acid	1.5	31	2	3	4
Tartaric acid	2.0	28	2	2	4
Tartaric acid	2.0	127	2	3	5
Tartaric acid	4.0	100	2	3	6
Benzoic acid	0.1	14	2	3	5
Benzoic acid	0.2	14	4	5	....
Benzoic acid	0.2	423	....	....	....
Benzoic acid	0.2	590	....	....	....
Benzoic acid	0.5	14	....	....	....
Benzoic acid	0.5	423	....	....	....
Benzoic acid	0.5	590	....	....	....
Sodium benzoate	0.1	14	2	3	5

Results of Germination Tests—Continued

Cultures from which inoculations were made. Development in normal solution.

Agents		Number of days in contact with agent	No. of days to		
Kind	Per cent		germinate	form conidia	mature
Sodium benzoate	0.2	14	2	3	5
Sodium benzoate	0.2	423	...	...	...
Sodium benzoate	0.2	590	...	...	...
Sodium benzoate	0.5	14	4	5	...
Sodium benzoate	0.5	423	...	...	...
Sodium benzoate	0.5	590	...	...	...
Boric acid	0.1	14	1	3	4
Boric acid	0.2	14	2	4	5
Boric acid	0.2	423	...	...	...
Boric acid	0.2	590	...	...	...
Boric acid	0.5	14	5	...	...
Boric acid	0.5	423	...	...	...
Boric acid	0.5	590	...	...	...
Borax	0.1	14	2	3	4
Borax	0.2	14	2	3	5
Borax	0.2	423	...	...	...
Borax	0.5	14	...	...	...
Borax	0.5	423	...	...	...
Borax	0.5	590	...	...	...
Formic acid	0.2	423	3	4	6
Formic acid	0.2	590	...	...	...
Formic acid	0.5	423	...	...	...
Formic acid	0.5	590	...	...	...
Formic acid	1.0	9	...	...	...
Formic acid	1.0	108	...	...	...
Sodium formate	0.2	423	3	7	9[1]
Sodium formate	0.2	590	...	...	...
Sodium formate	0.5	423	3	4	6
Sodium formate	0.5	590	...	...	...
Salicylic acid	0.1	14	1	3	4
Salicylic acid	0.2	14	1	3	...
Salicylic acid	0.2	423	5	7	12[2]
Salicylic acid	0.2	590	7	...	...
Salicylic acid	0.5	14	...	...	...
Salicylic acid	0.5	423	...	...	...
Salicylic acid	0.5	590	...	...	...
Sodium salicylate	0.1	14	1	3	4
Sodium salicylate	0.2	14	1	3	4
Sodium salicylate	0.2	423	3	4	6
Sodium salicylate	0.2	590	5	8	9
Sodium salicylate	0.5	14	1	3	5
Sodium salicylate	0.5	423	3	4	6
Sodium salicylate	0.5	590	4	5	7
Saccharin	0.1	14	2	3	4
Saccharin	0.2	14	2	2	4
Saccharin	0.2	423	3	4	6
Saccharin	0.2	590	3	7	13
Saccharin	0.5	14	1	2	4
Saccharin	0.5	423	3	4	6
Saccharin	0.5	590	5[3]	...	...
Saccharin	1.0	8	2	5	7

---

[1] Curled. [2] Surface yellow. [3] Wet curled masses on surface.

RESULTS OF GERMINATION TESTS—Continued

Cultures from which inoculations were made. Development in normal solution.

Agents		Number of days in contact with agent	No. of days to		
Kind	Per cent		germinate	form conidia	mature
Saccharin	1.0	107	3	5	8
Saccharin	1.0	590	5	...	...
Saccharin	2.0	21	3	7	8
Sodium sulphite	0.1	14	3	3	5
Sodium sulphite	0.2	14	2	2	5
Sodium sulphite	0.2	423	3	...	12[1]
Sodium sulphite	0.2	590	7	...	...
Sodium sulphite	0.5	14	...	...	...
Sodium sulphite	0.5	423	2	8	12[2]
Sodium sulphite	0.5	590	3	9	13[3]
Copper sulphate	0.1	14	3	4	5
Copper sulphate	0.2	14	2	3	4
Copper sulphate	0.2	423	2	3	4
Copper sulphate	0.2	590	7[4]	...	...
Copper sulphate	0.5	14	2	3	4
Copper sulphate	0.5	423	2	3	6
Copper sulphate	0.5	590	3	5	7
Copper sulphate	1.0	21	...	...	...
Copper sulphate	1.0	28	...	...	...
Copper sulphate	1.0	117	...	...	...
Alum	1.0	35	2	4	6
Alum	1.0	134	3	5	6
Creosote	0.02	28	2	2	4
Creosote	0.02	127	3	5	6
Creosote	0.04	25	2	3	4
Creosote	0.04	124	5	6	8
Formaldehyde (40 per cent)	0.02	14	2	2	3
Formaldehyde (40 per cent)	0.02	118	2	3	6
Formaldehyde (40 per cent)	0.04	19	2	2	4
Formaldehyde (40 per cent)	0.04	177	1	3	4
Formaldehyde (40 per cent)	0.1	8	...	...	...
Formaldehyde (40 per cent)	0.1	21	...	...	...
Formaldehyde (40 per cent)	0.1	28	...	...	...
Formaldehyde (40 per cent)	0.2	21	...	...	...
Formaldehyde (40 per cent)	0.2	28	...	...	...
Zinc chlorid	0.1	34	2	3	4
Zinc chlorid	0.1	132	3	5	7
Zinc chlorid	0.1	202	2	6	8
Zinc chlorid	0.2	34	2	3	4
Zinc chlorid	0.2	132	3	6	7
Zinc chlorid	0.2	202	3	4	6
Oxalic acid	0.5	28	2	3	4
Oxalic acid	0.5	127	2	5	6
Oxalic acid	0.5	197	2	3	5
Oxalic acid	1.0	25	2	3	4
Oxalic acid	1.0	124	3	6	7
Oxalic acid	1.0	194	2	6	8
Tannic acid	3.0	28	2	2	4
Tannic acid	3.0	127	3	5	7
Tannic acid	3.0	197	3	4	7
Carbolic acid	0.02	8	2	2	3
Carbolic acid	0.02	107	2	3	5
Carbolic acid	0.04	8	2	4	4
Carbolic acid	0.04	177	2	3	4

[1] Yellow. [2] Yellow. [3] Yellow. [4] Tiny compact submerged colonies.

Results of Germination Tests—Continued

Cultures from which inoculations were made. Development in normal solution.

Agents		Number of days in contact with agent	No. of days to		
Kind	Per cent		germinate	form conidia	mature
Carbolic acid	0.1	13	2	4	5
Carbolic acid	0.1	21	....	....	....
Carbolic acid	0.1	127	....	....	....
Carbolic acid	0.1	176	....	....	....
Carbolic acid	0.2	21	....	....	....
Carbolic acid	0.2	127	....	....	....
Hydrochloric acid	0.5	39	1	2	4[1]
Hydrochloric acid	0.5	138	3	8	10
Nitric acid	0.2	138	2	3	5
Nitric acid	0.5	32	4	11	13[2]
Sulphuric acid	0.1	34	2	2	4
Sulphuric acid	0.1	132	3	4	8
Sulphuric acid	0.2	32	4	10	12
Sulphuric acid	0.5	21	....	....	....
Potassium hydrate	0.2	14	2	2	4
Potassium hydrate	0.2	113	2	4	5
Potassium hydrate	0.5	19	2	3	4
Potassium hydrate	0.5	118	2	5	6
Potassium hydrate	1.0	12	3	7	9
Potassium hydrate	1.0	118	....	....	....
Potassium hydrate	1.0	188	....	....	....
Sodium hydrate	0.2	14	2	2	4
Sodium hydrate	0.2	113	2	4	6
Sodium hydrate	0.5	19	2	3	6
Sodium hydrate	0.5	118	6	8	10
Sodium hydrate	0.5	188	7	8	....
Sodium hydrate	1.0	12	....	....	....
Sodium hydrate	1.0	19	....	....	....
Atropin sulphate	0.0065	31	2	3	4
Atropin sulphate	0.0065	130	3	5	6
Cocain hydrochlorate	0.1625	31	2	3	4
Cocain hydrochlorate	0.1625	130	2	4	6
Morphin sulphate	0.1625	31	2	3	4
Morphin sulphate	0.1625	130	3	6	7
Strychnin nitrate	0.0065	31	2	2	3[1]
Strychnin nitrate	0.0065	130	3	5	6
Quinin sulphate	0.2	34	2	3	4
Quinin sulphate	0.2	132	2	5	6
Quinin sulphate	0.5	34	2	5	6
Quinin sulphate	0.5	132	2	5	7
Mercuric chlorid	0.01	28	2	2	4
Mercuric chlorid	0.01	127	3	5	7
Mercuric chlorid	0.1	8	4	9	10
Mercuric chlorid	0.1	107	....	....	....

[1] Heavier than control. [2] Surface rough.

As in the previous experiments, the microscopic characters were of greater value than the macroscopic in determining the deviations from the normal which appeared in the following generation. In many cases where growth appeared to be normal, the minute structure showed more or less variation, similar to that induced in the organism when in contact with the agent. That the changes produced by some of the agents were of a vital character and sufficiently pronounced to cause the protoplast to exceed the limit of physiological elasticity is evidenced by their transmission to a greater or less extent to the following generation. It seems possible that if the action of the inducing agent were continued, the changes produced might ultimately become permanent, if they did not finally destroy the organism, since changes of a less violent character disappear when the organism is returned to the original conditions, according to Thom.[1] His statement which is based on extensive cultural work is "Many species differ so materially in gross characters when grown upon these different media that successive cultures, if not known to be pure transfers, might be supposed to be different species, but when returned to the original media and conditions these forms have immediately produced the characters and reactions first found, with a large degree of uniformity." It is probable, however, that with the development of the organism in a normal environment, the variations would gradually disappear, and the organism assume its original form and structure, since according to Pfeffer,[2] variations of this nature being labile only gradually acquire a fixed hereditary character. In the cases in the original experiments, where the most pronounced effects of the agents, swelling and various degrees of disorganization, took place, similar variations were present in the following generation, these being distinct from the changes resulting from the reduced vitality due to the age of the inoculating spores. In those experiments in which the agents merely reduced the food supply of the organism, the following generation was practically normal, no results other than those seen in the control being evident except in the case of the 15 per cent salt culture, in which the characters were apparently transmitted.

The results of the experiments indicate that the organism when in a labile condition is susceptible to the influence of external conditions, which may account to a certain extent for many of the so-called species of this genus. They also indicate that the organism possesses great adaptability and resistance to variable and adverse conditions shown in the ability to develop after having been subjected to the influence of the many diverse and deleterious substances, some so strong as to prevent development while the conidia remained in contact with them, others, though permitting a retarded germination, producing extreme changes in structure and form.

See Plates 58–62.

GENERAL CONSIDERATIONS

The cultures of the organisms in which the various chemicals were used were made, as nearly as possible, under similar conditions. The stock medium, an organic solution, was kept at uniform acidity; the containers were flasks of the same size, so that there were equal surface areas of the solution; the same amount of solution, 100 c.c., was used in all, so as to avoid any variations due to the number of spores present in a given volume of the medium; the cultures were kept shaded, and at an approximately even temperature. The method of seeding has been described, and was as nearly uniform as possible.

Preliminary to the work with the agents, the moulds were grown in moist chambers, with air and without, to determine the effect of a lack of oxygen on development. The result of the lack of oxygen, but sufficient food supply, was a reduction of the protoplasm, and an increase in the number of septa, the cells thus formed becoming rounded[1] in many cases. There was an increase in the formation of fruiting in the Penicillium and a greater degree of catenulation in the Alternaria. It is stated by Pfeffer[2] that P. glaucum is destroyed or severely injured by the withdrawal of oxygen for a single hour. The effort in this work was to determine the result of other factors apart from the agents in producing morphological changes in the organisms.

---

[1] Cultural Studies of Species of Penicillium, U. S. Dept. of Agr., B. A. I., Bul. 118, 1910.

[2] Physiology of Plants, Vol. 3, 1903.

The macroscopic appearance of the growths of the organisms in the respective solutions though indicating by retardation and abnormalities of development the general effect of the agents on the structure, is not to be depended upon alone for an accurate estimation of the effect. In some cases where a seemingly normal growth occurred, the microscopic appearance showed the most pronounced effect in the swelling, distortion, and disorganization of the hyphae, whereas in other cultures with the same agent in which the growth was stunted, the microscopic appearance indicated a nearly normal development, as if the mould had offered a greater resistance to the specific action of the agent.

In noting the effects of the various agents on the histological structure, it was found that an approximate classification might be made. The effects induced were plasmolysis, stunting, distortion, hardening of the protoplasm, reduction of the protoplasm to a comparatively small number of refractive granules, disorganization of the protoplasm, disorganization of the cell-walls, the increased formation of septa thus forming abnormally short cells, many short sub-branches, sub-branches developed into more or less stunted fruiting heads in the Penicillium, and with some agents a combination of different effects. The disorganization of the protoplasm produced a granulation, more or less coarse, with no distinction between the ectoplast and endoplast. This lack of differentiation gave a swollen appearance to the hyphae, though in some cases they were less than the normal size, there were no vacuoles, and apparently little cohesion. The disorganization of the walls was evidenced by their breaking from the weight of the cover-glass or in the transference of the hyphae from the culture into a drop of the culture medium on a slide, and complete disruption when placed in a drop of distilled water.

The salt, sugar, and potassium nitrate produced plasmolysis resulting finally in complete arrest of growth from starvation. That the organism was not killed was shown by the germination tests with the Penicillium. The effect was really a "physiological drought"[3] produced by a high concentration of a non-toxic salt, which agrees with the statement of Pfeffer[4] that when the osmotic concentration of the culture fluid passes a certain limit, growth becomes impossible, though no poisonous effect is exercised.

The majority of the spices were innocuous, producing neither retardation nor abnormal effects, so far as could be ascertained from their gross development and microscopic appearance. Those which possessed antiseptic properties—allspice, cinnamon, and cloves—produced varying degrees of enlargement, accompanied by disorganization of the protoplasm and walls. The mustard produced starvation and also disorganization of the protoplasm, as indicated by the stunted and distorted hyphae, the swollen and non-hyaline appearance of the protoplasm, and the tendency in the Penicillium to form fruiting heads almost directly from the hyphae developed from the germinated spore. The hyphae formed matted tufts, difficult to separate, and with blunt distorted fruiting heads. A peculiarity of the mustard was that the active principle causing the abnormal growth produced the effect when present in minute quantities, as in the 0.1 per cent solution of the ground mustard and the 1 per cent of seeds. Stevens[1] working on P. crustaceum states that "peculiar knotted or twisted hyphae frequently result from the attempt to grow in a poisonous solution." Clark used P. glaucum and found that the effect of deleterious agents on the mycelium was "varied and often characteristic."

---

[1] L. Pasteur: Studies on Fermentation, trans. by F. Faulkner: 105, 1879.
[2] *Loc. cit.*, Vol. I: 536.
[3] Osterhout: *loc. cit.*
[4] *Loc. cit.*, 421.

The ordinary acids—citric, lactic, and malic—which are present in fruits and vegetables, caused only a slight retardation in development when as large an amount as 6 per cent was used, which is more than occurs in fruits other than the lemon. More than this amount apparently checked metabolism, as shown in the reduced and impoverished appearing protoplasm. The tartaric acid was much stronger, the 4 per cent solution, while only slightly retarding development, caused stunting, lesser amounts causing swelling and disorganization. The other organic acids—acetic, benzoic, boric, butyric, and salicylic—and their sodium salts caused a complete disorganization of both the protoplasm and walls. There was a decided retardation of development by even small amounts, and in some of them a yellowing or browning of the protoplasm. In the extreme stages the hyphae were much swollen, and filled with a coarsely granular, non-hyaline mass, showing no distinction of ectoplast, and on rupture, spreading as a disintegrated mass.

The salts of the metals produced much the same results, though not so extreme in either swelling or disorganization, and with amounts that were not so close to the inhibitive points. When the larger amounts were used, and germination much delayed, stunted, curled colonies were developed with attenuated hyphae, and apparently coagulated protoplasm. Similar changes resulted with the lesser amounts when germination was delayed by low temperature.

Swelling by the lesser amounts, and stunting by the greater were also produced by carbolic and the mineral acids, creosote, mercuric chlorid, and the alkaloids. Mercuric chlorid and the alkaloids caused disorganization of the protoplasm, shown at first by the full, granular, non-hyaline appearance with no distinction of limiting membrane and plasma, followed by the reduction of the plasma to a thin layer, containing few granules.

The alcohol retarded development, producing swelling, accompanied by distortion and hardening; in some cases a shrinking without distortion occurred, and in others disorganization.[2]

There were also some peculiar effects, such as the formation of fruiting heads in the Penicillium, formed directly from the primary hyphae and in the Alternaria a long chain of conidia, the formation of an abnormally large number of septa, the formation of giant cells, and colonies that bore no resemblance to the ordinary development. In some of the badly distorted colonies which were firm and difficult to separate, and were dry masses on the surface of the solution, after a time drops of brownish liquid appeared in the depressions, though the solution was pale amber.

---

[1] *Botanical Gazette,* Dec., 1898.

[2] Dujardin—Beaumetz and Audigé: Experimental researches on the toxic power of the alcohols. 1879.

In estimating the effect of the respective chemicals upon the living cell or protoplast, it is necessary to consider the condition, or vital activity, of the organism, as well as the external conditions which act as indirect agents in inducing changes. It could not be determined how far the chemicals used were able to penetrate the cell substance in their original condition, since plants absorb all[1] substances which are in solution whether useful or not, though some of these may penetrate only through the cell wall, and not into the living plasma. This is true of some salts which plasmolyze the cell, accumulating in the space between the cell wall and the protoplasm. This can be seen readily if colored solutions are used, as with sugar, salt, or potassium nitrate colored with aniline blue or beetroot juice. All normal cells are in a state of turgor, which is maintained by substances in solution within the cells which are incapable of diosmosis. These substances are such as to cause the limiting layer of the protoplast to press with sufficient force against the cell wall to stretch it to some extent. When the cell is plasmolyzed, the protoplast contracts until the osmotic solutions become equalized, which may take place rapidly in the case of the absorption of the plasmolyzing agent. In order to be absorbed by the plasma the substance must not only pass through the cell wall, but also through the limiting membranes of the plasma. It has been determined by Pfeffer that all substances, useful, unnecessary, or even poisonous, which pass through the limiting membranes penetrate the protoplast. Among the substances which it has been demonstrated enter the protoplast are free acids, carbon dioxid, the caustic alkalis, iodin, and mercuric chlorid,[2] the first if used for a short time and in dilute solution, being harmless, whereas, the iodin and mercuric chlorid are decidedly injurious. Some substances cause a precipitate to form in the cells, while still others are accumulated to such an extent as to change the characteristic properties of the cells. Where the absorbed substances cause a chemical action to take place within the cells, the equilibrium between the internal and external solutions is constantly changing with a consequent continuity of absorption, so that the plant may absorb a relatively large quantity of a substance from a dilute solution, or in the case of a mixture of substances, one may be absorbed in much larger amounts than the other. These facts might explain some of the peculiar phenomena obtained in the tests in which a very small amount of the chemical used produced results commensurate with those obtained from tests in which much larger amounts were employed, and also some of the results obtained in the tests with mixtures. The limiting membranes also may be modified so as to exercise a regulatory function in regard to the substances absorbed, absorption being greater at one time than another, as shown in the more pronounced effect of a substance on some parts of the plant than on others and also at different periods.

---

[1] Pfeffer, *loc. cit.*, p. 86.

[2] Pfeffer, *loc. cit.*, p. 98.

LITERATURE

C. L. Alsberg and O. F. Black: "Contributions to the study of maize deteriorations." Bul. 270, B. P. I., Dept. of Agr.

F. M. Bachmann: "The inhibiting action of certain spices on the growth of microorganisms." Jour. Ind. Chem., Vol. 8 (1916), No. 7:620-623.

C. Brooks: "Temperature and toxic action." Bot. Gaz., 42:359-375, 1906.

C. M. Buchanan: "Antisepsis and Antiseptics." 1895.

J. F. Clark: "On the toxic effect of deleterious agents on the germination and development of certain filamentous fungi." Bot. Gaz., 28:289, 375, 1899.

B. M. Duggar: "Fungous diseases of plants." New York, 1909.

Dugardin-Beaumetz and Audige: "Experimental researches on the toxic power of the alcohols." Paris, 1879.

N. Gamaleia: "The Bacterial Poisons." Trans. by E. P. Hurd, 1893.

C. H. Gill: "British Manufacturing Industries." 1876.

K. E. Golden: "Salt as a deterrent in yeast fermentation." Proc. A. A. A. S., 1898.

H. Hasselbring: "The carbon assimilation of Penicillium." Bot. Gaz., 45:176-193, 1908.

O. Hertwig and H. J. Campbell: "The Cell." New York, 1895.

A. Herzen: Deut. Chem. Ges., Ber. 8:822.

J. W. Holland: "Medical Chemistry and Toxicology." Philadelphia, 1905.

H. C. Irish: "Revision of the genus Capsicum." 9th Ann. Rep. Missouri Bot. Gar., April, 1898.

E. O. Jordan: "General Bacteriology." 1916.

E. Kayser: "Les levures." Paris.

F. Lafar: "Technical Mycology." Vol., 2, part 1, 1903.

H. Leffmann and W. Beam: "Select Methods in Food Analysis." 1905.

J. Loeb: "Popular Science Monthly." 80:1, 1912.

K. P. McElroy and W. D. Bigelow: "Foods and Food Adulterants." U. S. Dept. of Agr., Bul. 13, Part 8, 1893.

M. M. McCool: "The action of certain nutrient and non-nutrient bases on plant growth." Cornell Univ. Ag. Exp. Sta., Memoir No. 2, 1913.

W. J. V. Osterhout: Bot. Gaz., 47:148-149, 1908.

C. J. Overman and L. E. Sayer: "Merck's report." 6:279, 1897.

L. Pasteur and F. Faulkner: "Studies on Fermentation." London, 1879.

A. H. Peck: Jour. Am. Med. Assn., 32:6-11, 1899.

W. Pfeffer and A. J. Ewart: "Physiology of Plants." Oxford, 1906.

J. Reichel: "The behavior of Penicillium with acetic acid and its salts." Ztschr. 30 (1910), Nos. 1-2: 152-159. (Abs. in Ex. Sta. Rec., Vol. 26: 203.)

H. M. Robinson and C. H. Cribb: "The Law and Chemistry of Food and Drugs." 1895.

A. Rolet: "Les Conserves de Fruits." 1912.

J. de Rufz: "Penetration of salts into the protoplasm and the nature of the toxic action." Ann. Sci. Nat. Bot. (9 ser.), 14: 97-189.

H. Schroeter: "Pure Products." 1908, 4:199.

F. L. Stevens: "The fungi which cause plant disease." New York, 1913.

F. L. Stevens and J. C. Hall: Bot. Gaz., pp. 1-30, 1909.

C. Thom: "Cultural studies of species of Penicillium." Bul. 118, B. A. I., Dept. of Agr.

E. L. Trouessart: "Antiseptic Therapeutics." Paris. Translated by E. P. Hurd, 1893.

H. J. Waterman: "Mutation in Penicillium glaucum and Aspergillus niger." Ztschr. Garungsphysiol., 3 (1913), No. 1: 1-14. (Abs. in Exp. Sta. Rec., Vol. 31 (1914): 225.)

——————: "U. S. Dispensatory," 19th Ed.

——————: "Food Inspection Decision," No. 126, 1910.

L. Pasteur: "Studies on Fermentation." Translated by F. Faulkner and D. C. Robb, 1879.

W. Pfeffer: "Physiology of Plants." Translated by A. J. Ewart, 1900.

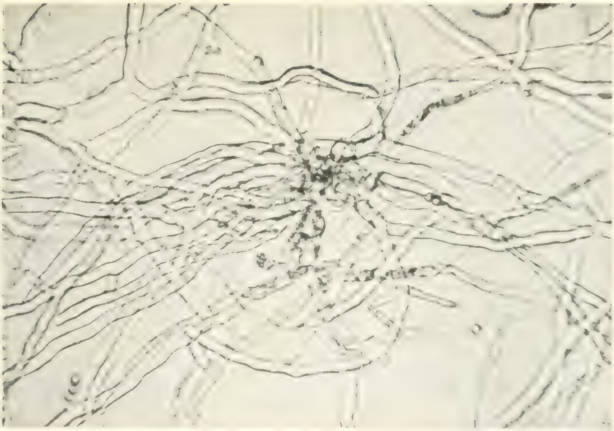
EXPLANATION OF PLATES

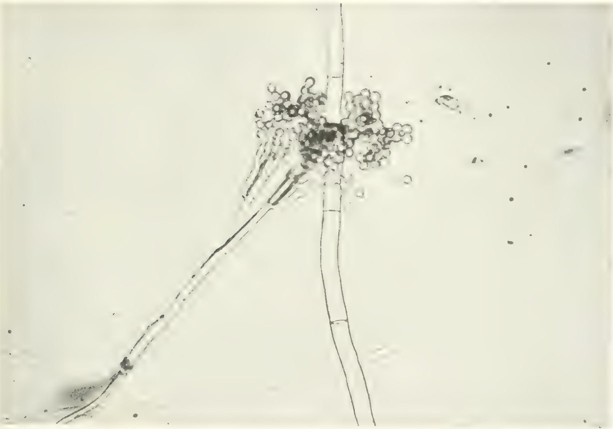
The illustrations are of the living plants, and taken as soon after germination as there was sufficient development to show the characteristic effect of the agent. Each mount was made in a few drops of the medium in which it had grown and was transferred from the flask to the slide by means of a platinum loop. The magnification is X 400. A Zeiss photomicrographic stand No. 1c was used and a Zeiss vertical camera.

PLATE I

Control.

1. Penicillium, normal hyphae, showing germinated conidia and branching hyphae.
2. Penicillium, normal fruiting head.
3. Alternaria, showing germinated conidium and hyphae.





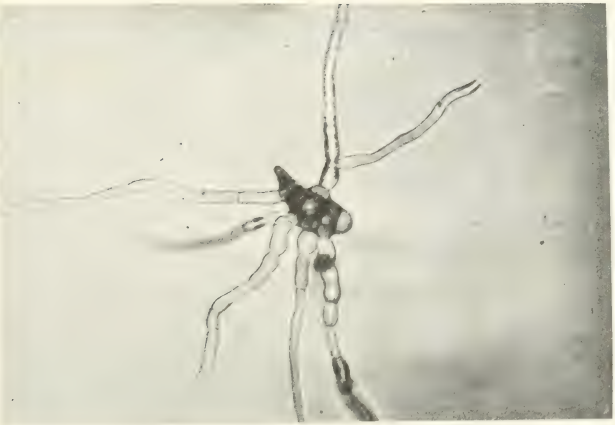
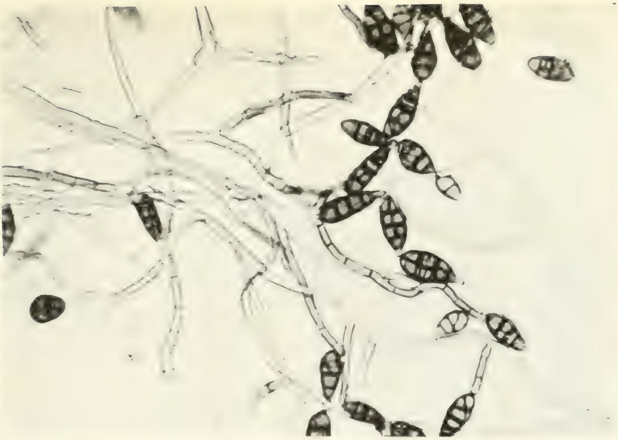
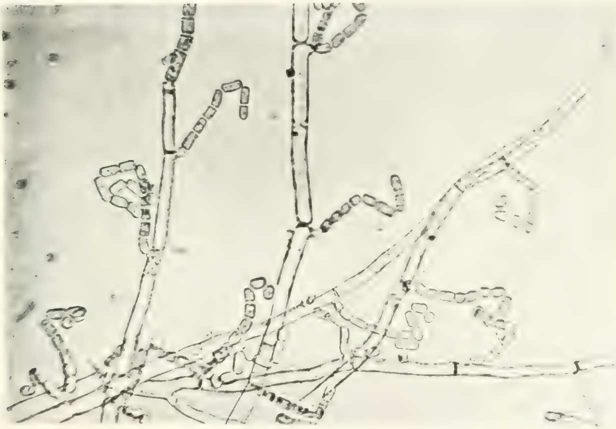


PLATE II

Control.

1. Alternaria, conidia.
2. Oidium, normal hyphae and oidia.
3. Oidium, normal development, showing profuse formation of oidia.





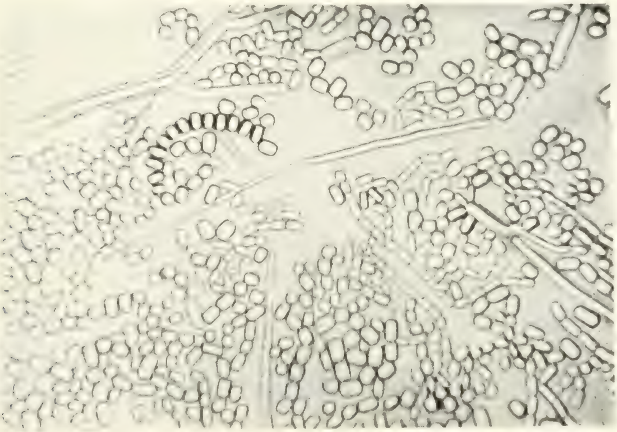
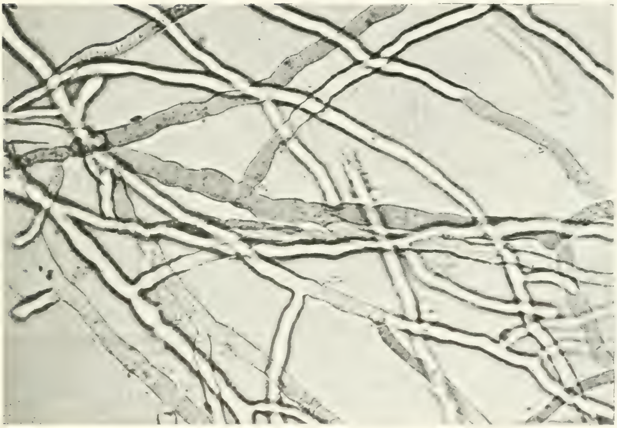


PLATE III

Salt (sodium chloride).

1. Penicillium, showing development of mycelium in 15 per cent solution.
2. Alternaria, showing development of mycelium in 15 per cent solution.
3. Oidium, showing development of hyphae and oidia in 10 per cent solution.



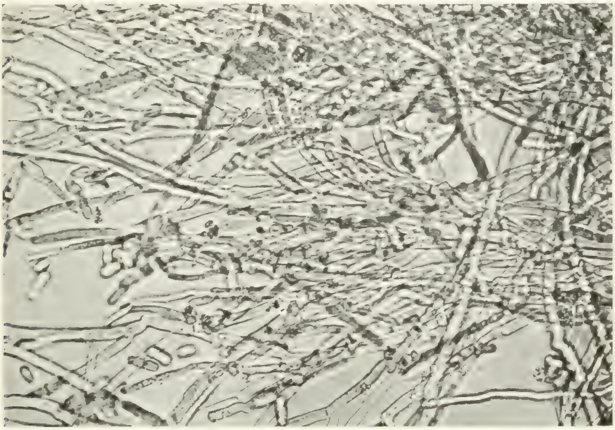


PLATE IV

Salt (sodium chloride).

1. Penicillium, showing development in 5 per cent solution, hyphae swollen.
2. Alternaria, showing development in 5 per cent solution, germinated conidium and hyphae swollen.
3. Oidium, showing development in 5 per cent solution, slight swelling, also showing the clearing effect to better advantage than in the previous pictures, but which was noticeable in all.

PLATE V

Sugar.

1. Penicillium, showing development in 66⅔ per cent solution, showing stunting and distortion. In smaller amounts the hyphae were vacuolated as in the Alternaria.
2. Alternaria, showing development in 50 per cent solution, the hyphae having vacuoles at fairly regular intervals.
3. Oidium, showing development in 50 per cent solution. In smaller amounts the hyphae were vacuolated as in the Alternaria, and not swollen.

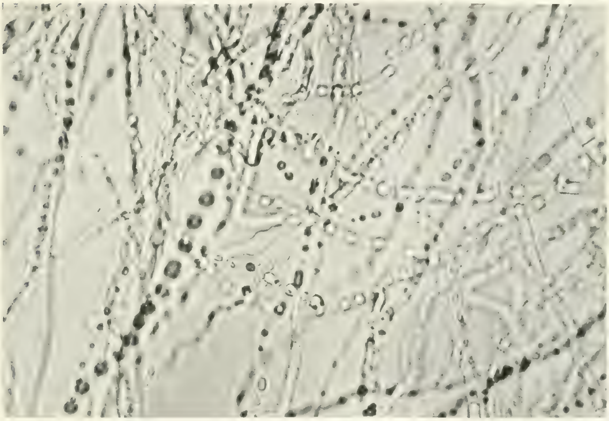


PLATE VI

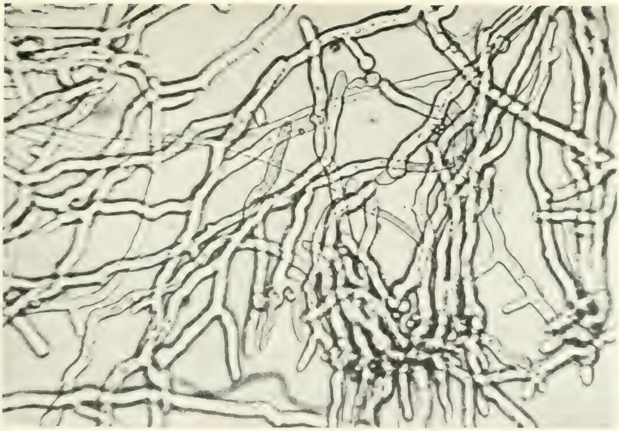
Saltpeter (potassium nitrate).

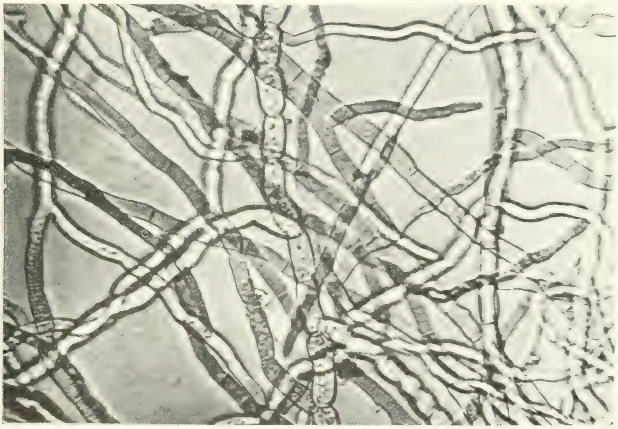
1. Penicillium, showing development in 25 per cent solution, crystals of the salt separated out of the solution.
2. Alternaria, showing development in 20 per cent.
3. Oidium, developed in 20 per cent solution, showing some swelling and distortion.

PLATE VII

Saltpeter (potassium nitrate).

1. Penicillium, growth in 10 per cent solution.
2. Alternaria, growth in 10 per cent solution.
3. Oidium, growth in 10 per cent solution.
   In the smaller amounts of the salt, the development of the three organisms was more nearly uniform.





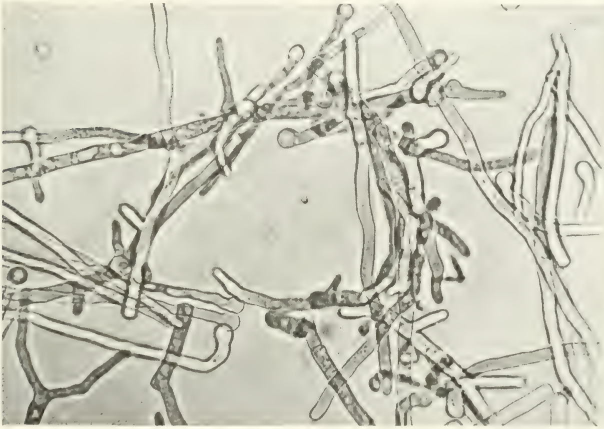


PLATE VIII

Allspice.

1. Penicillium, showing development in 2 per cent solution.
2. Alternaria, showing development in 5 per cent solution.
3. Oidium, showing development in 10 per cent solution.

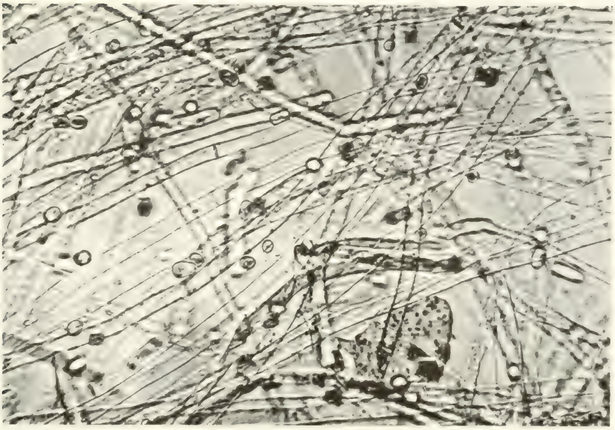


PLATE IX

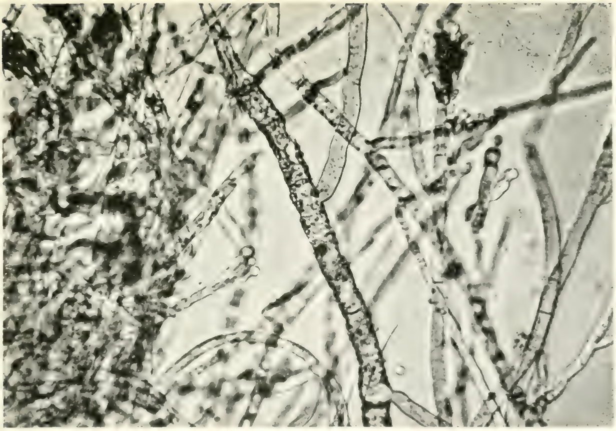
Allspice.

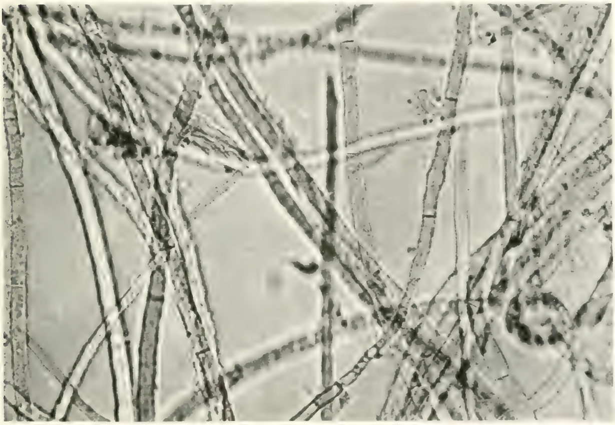
1. Penicillium grown in 1 per cent solution, showing stunted fruiting heads. The Penicillium was more sensitive than the other organisms to the action of the active principle of the allspice.

PLATE X

Cinnamon.

1. Penicillium, showing development in 10 per cent solution, the hyphae swollen and protoplasm disorganized.
2. Alternaria, grown in 10 per cent solution, showing disorganization.
3. Oidium, grown in 10 per cent solution, showing swelling and disorganization. A precipitate was formed in the solution which shows as a flocculence adhering to the hyphae.





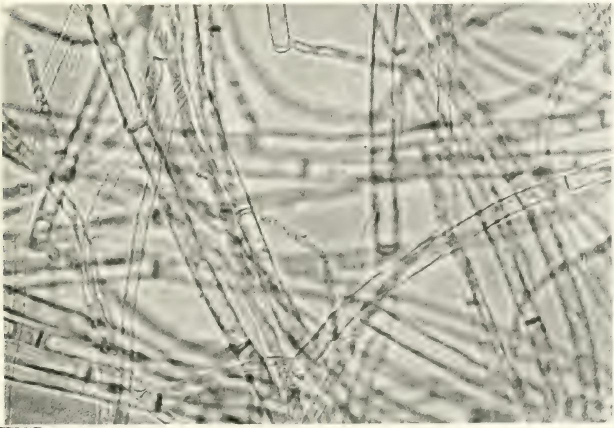


PLATE XI

Cloves.

1. Penicillium, 10 per cent solution, showing disorganized protoplasm.
2. Alternaria, 10 per cent solution, showing coarsely granular protoplasm.
3. Oidium, 20 per cent solution, showing coarsely granular protoplasm.

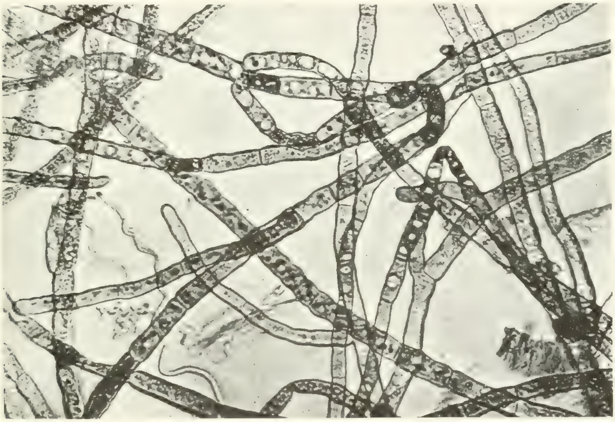
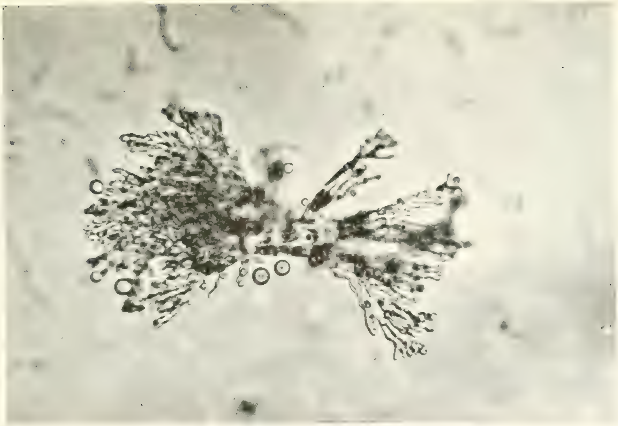


PLATE XII

Mustard.

1. Penicillium, ½ per cent solution, showing group of distorted fruiting heads, developed from the primary hyphae
2. Alternaria, 1 per cent solution, showing hyphae with disorganized protoplasm.
3. Oidium, 1 per cent solution, showing coarse granules in some hyphae and oidia. The Oidium showed the least susceptibility to the action of the mustard.



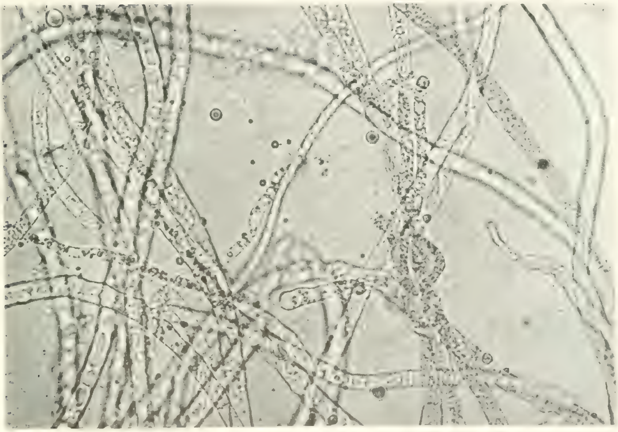
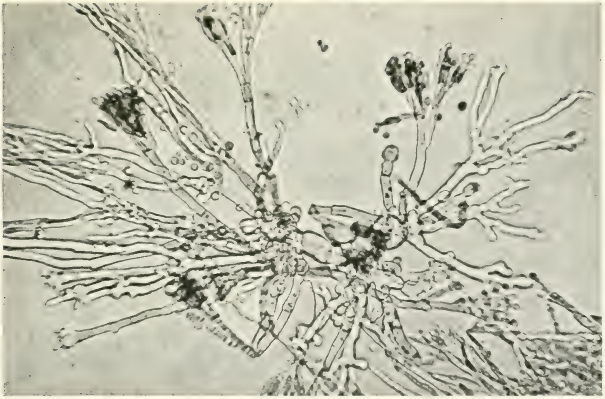
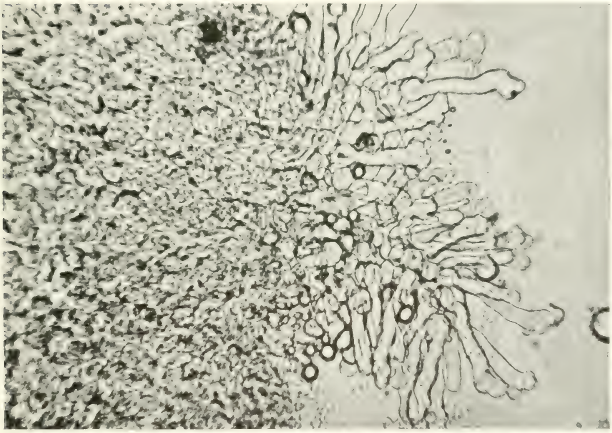


PLATE XIII

Mustard.

1. Penicillium, grown in a solution containing 25 per cent whole white mustard seed, showing characteristic effect.
2. Penicillium, grown in a solution containing 25 per cent whole black mustard seed, also showing characteristic bunching and distortion.
3. Alternaria, grown in 1 per cent solution, showing effect on conidia.





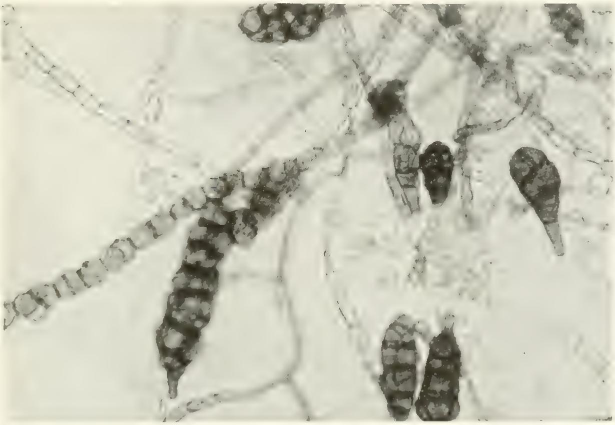


PLATE XIV

Acetic acid.

1. Penicillium, group of swollen and disorganized hyphae grown in 1 per cent acetic acid.
2. Alternaria, disorganized hyphae grown in 0.04 per cent acetic acid. The tips of the hyphae are ruptured, and the protoplasm flowing out.
3. Oidium, weakened and disorganized hyphae grown in 0.5 per cent solution.

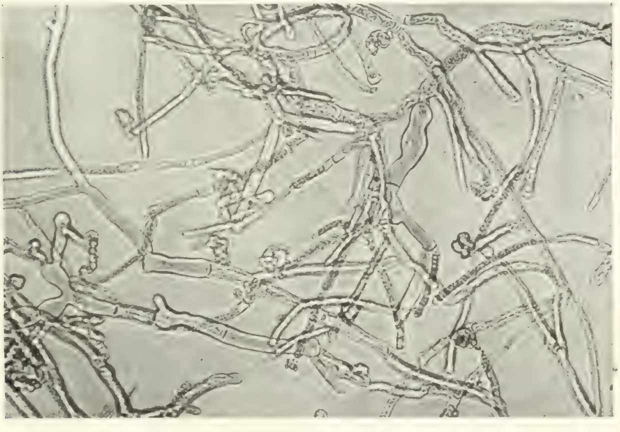
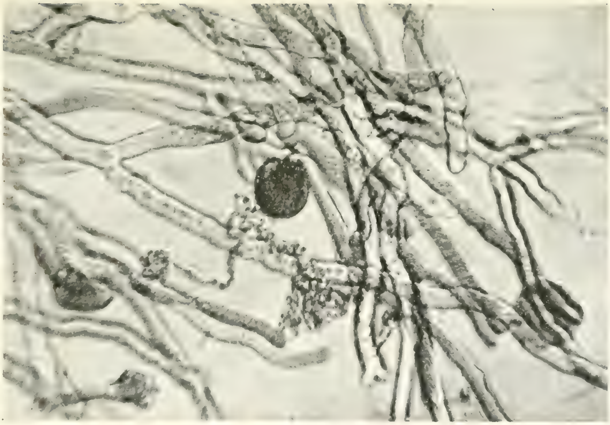


PLATE XV

Vinegars.

1. Penicillium grown in 1 per cent cider vinegar solution.
2. Penicillium grown in 1 per cent distilled vinegar.
3. Penicillium grown in 1 per cent malt vinegar.



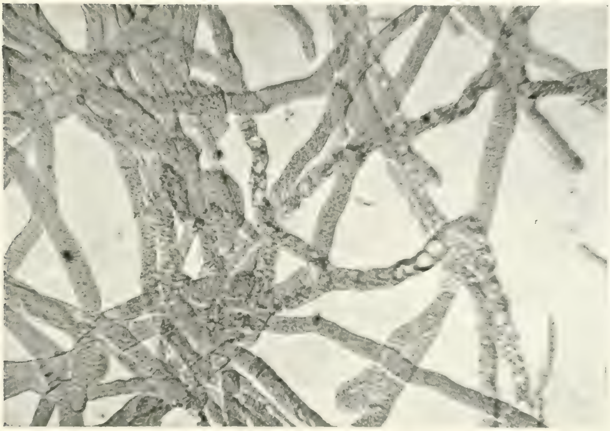


PLATE XVI

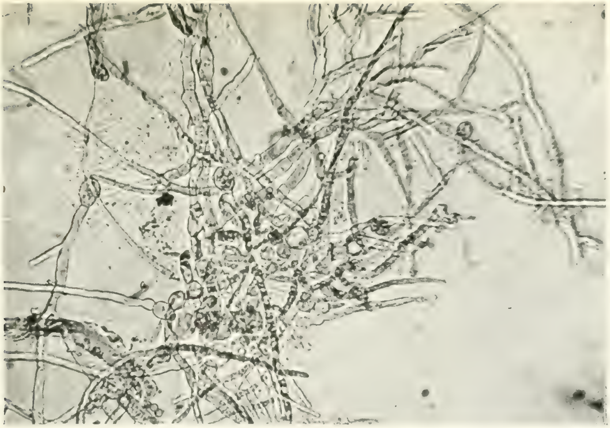
Butyric acid.

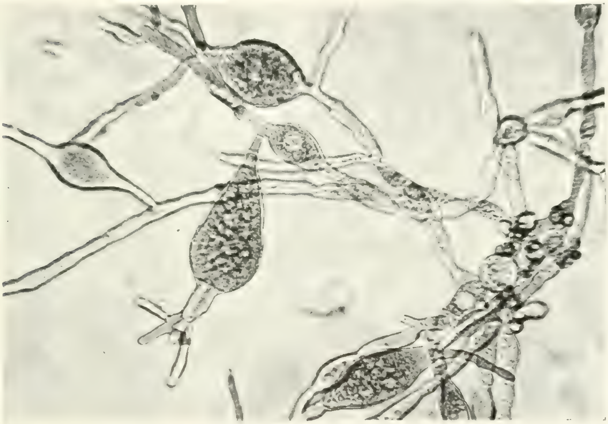
1. Penicillium, hyphae grown in 0.2 per cent solution, showing marked disorganization.
2. Alternaria, hyphae from 0.05 per cent solution, showing disorganized protoplasm.
3. Oidium, hyphae from 0.1 per cent solution, showing disorganized protoplasm.

PLATE XVII

Organic acids.

1. Penicillium grown in 10 per cent citric acid solution, showing disorganization of plasma and increased number of septa.
2. Penicillium grown in 10 per cent lactic acid solution, showing disorganization of plasma, "giant" cells, and peculiar branching.
3. Penicillium grown in 10 per cent malic acid, showing increased number of septa and some disorganization and stunting.





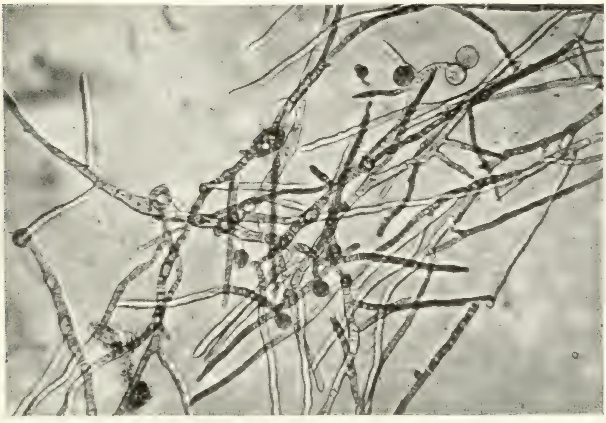
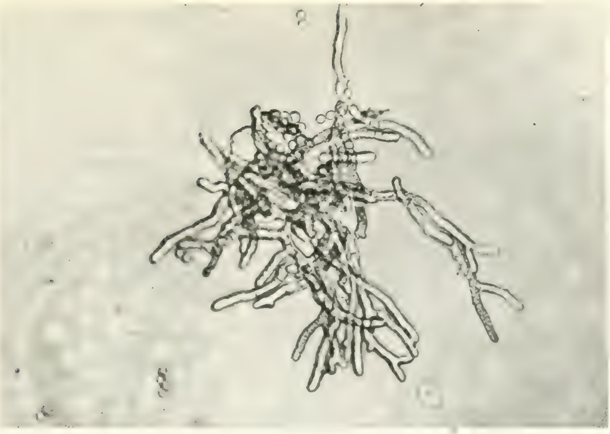
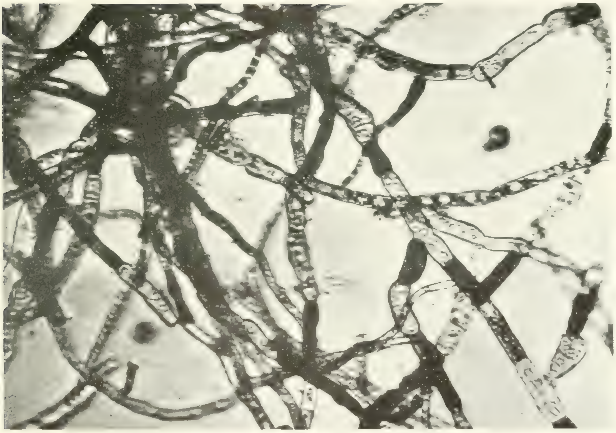


PLATE XVIII

Tannic acid.

1. Penicillium grown in 3 per cent solution, showing stunting and distortion.
2. Alternaria grown in 0.05 per cent solution, showing some stunting and coarse granules in the plasma. The Alternaria was very sensitive to the action of the acid.
3. Oidium grown in 0.5 per cent solution, showing stunting and disorganized plasma.





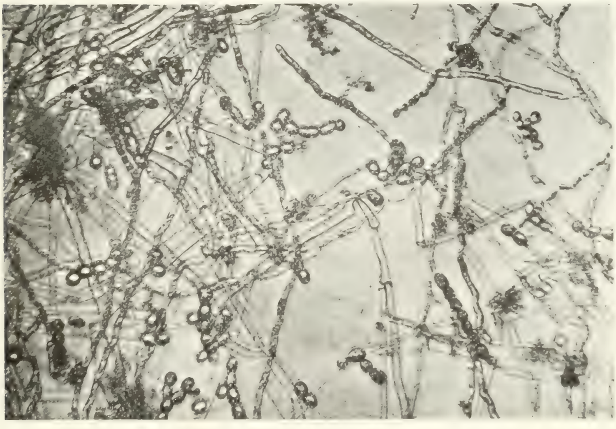


PLATE XIX

Tartaric acid.

1. Penicillium grown in 4 per cent solution, showing stunting.
2. Oidium grown in 3 per cent solution, showing coarse protoplasm.

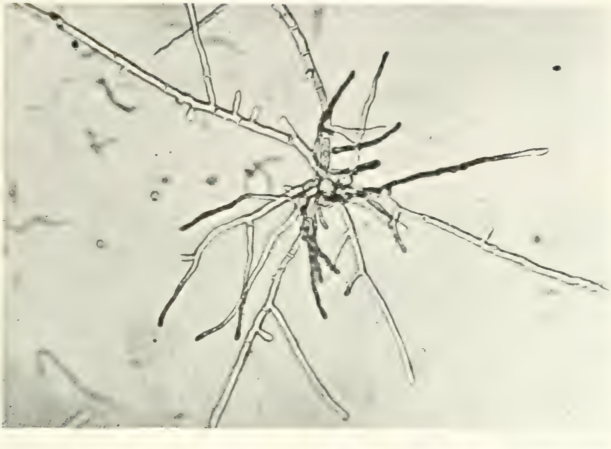
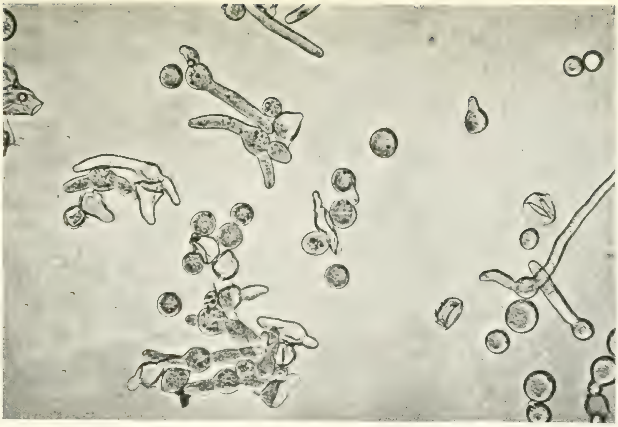
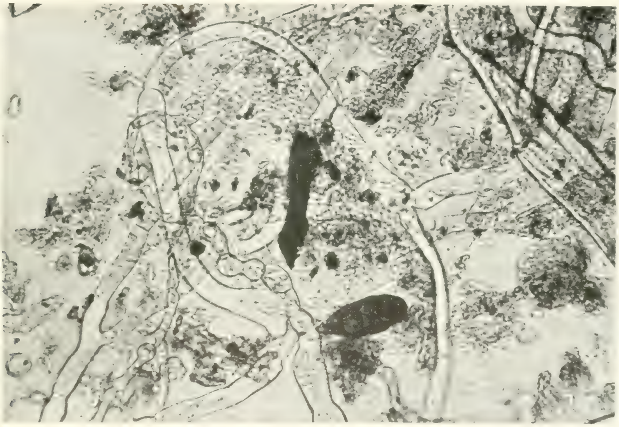


PLATE XX

Alcohol.

1. Penicillium, germinated conidia from 9.6 per cent solution, showing coarse granulation.
2. Alternaria, hyphae grown in 5 per cent solution, showing coarse granulation.
3. Oidium, hyphae grown in 5 per cent solution, showing swelling.





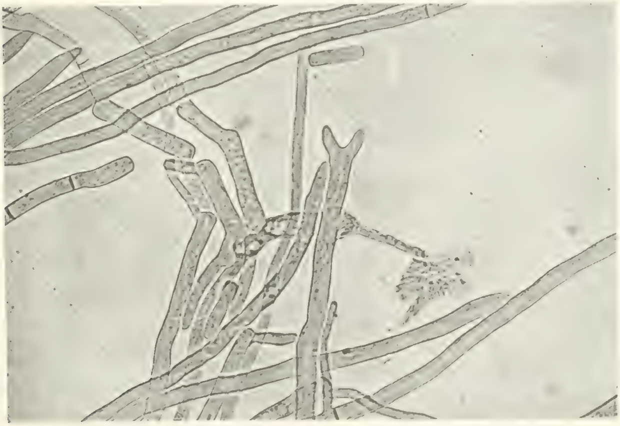


PLATE XXI

Benzoic acid.

1. Penicillium, hyphae grown in 0.2 per cent solution, showing swelling and disorganization.
2. Alternaria, mycelium from 0.02 per cent solution, showing swelling of some parts of hyphae and disorganization.
3. Oidium, hyphae and oidia from 0.02 per cent solution, showing disorganization.

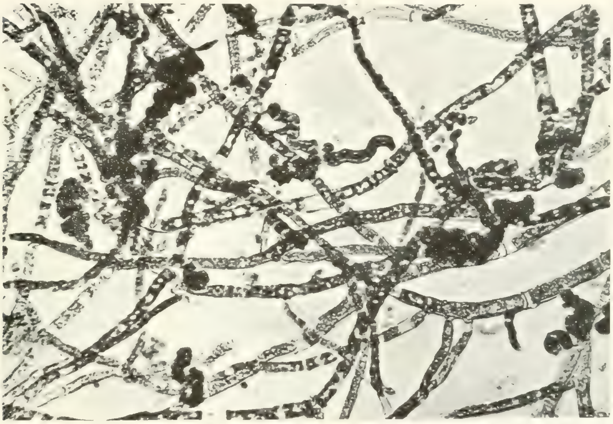
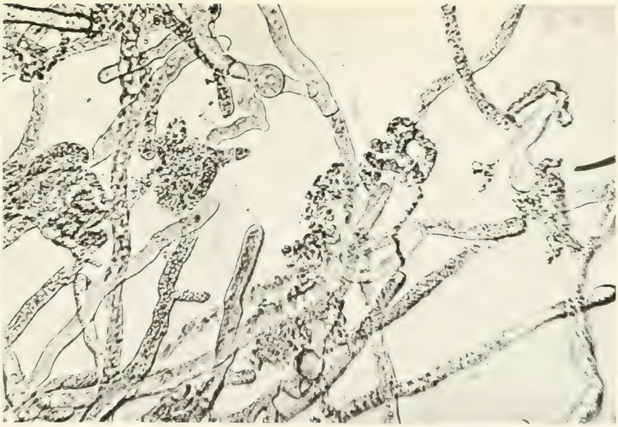
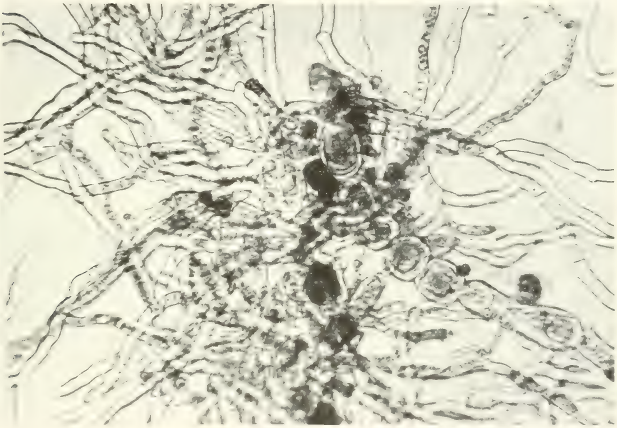


PLATE XXII

Sodium benzoate.

1. Penicillium, mycelium grown in 0.1 per cent solution, showing similar results to those resulting from the benzoic acid.
2. Alternaria, mycelium from 0.01 per cent solution, showing swelling and disorganization.
3. Oidium, mycelium from 0.03 per cent solution, practically the same result as produced by the 0.02 per cent acid solution.





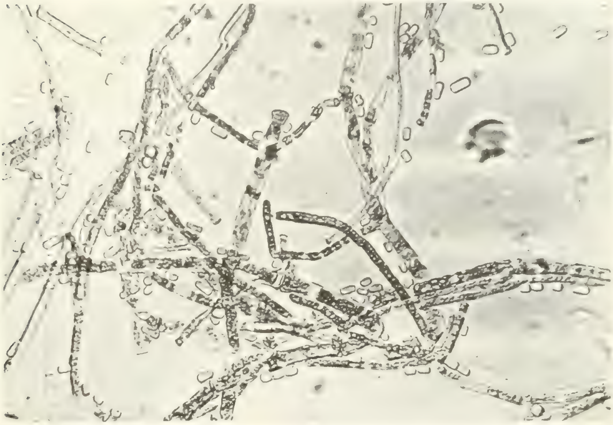


PLATE XXIII

Boric acid.

1. Penicillium, mycelium grown in 0.2 per cent solution, showing swelling and disorganization of plasma.
2. Alternaria, mycelium grown in 0.5 per cent solution, showing swelling and disorganization.
3. Oidium, germinated oidia and hyphae from 0.2 per cent solution, showing swelling and reduction of protoplasm.

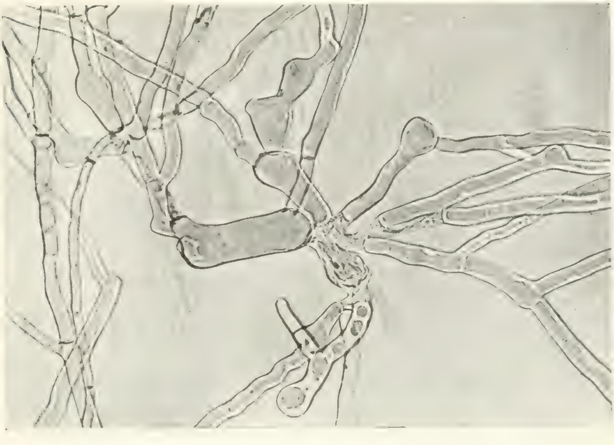
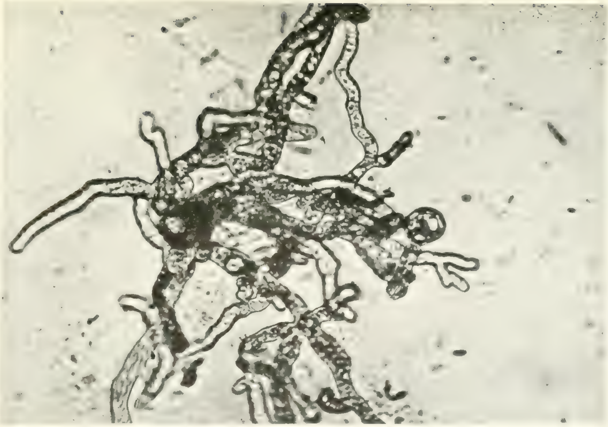
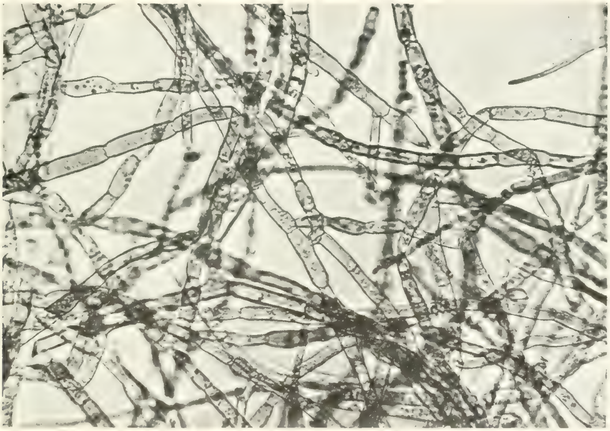


PLATE XXIV

Borax.

1. Penicillium, mycelium grown in 0.5 per cent borax, showing swelling and distortion.
2. Alternaria, mycelium from 0.5 per cent solution, showing protoplasm reduced and with coarse granules.
3. Oidium, mycelium and oidia from 0.5 per cent solution, showing hyphae weakened with inter-growth in one.





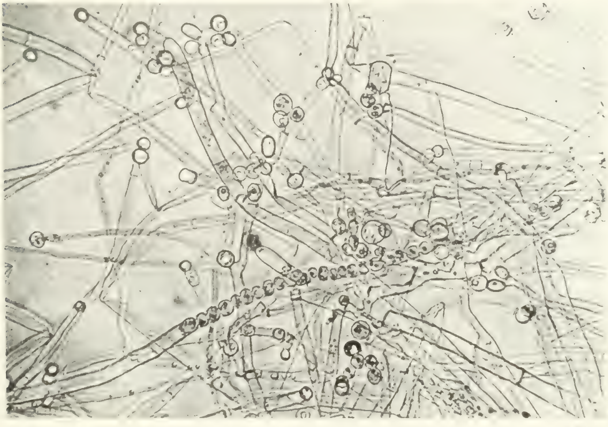
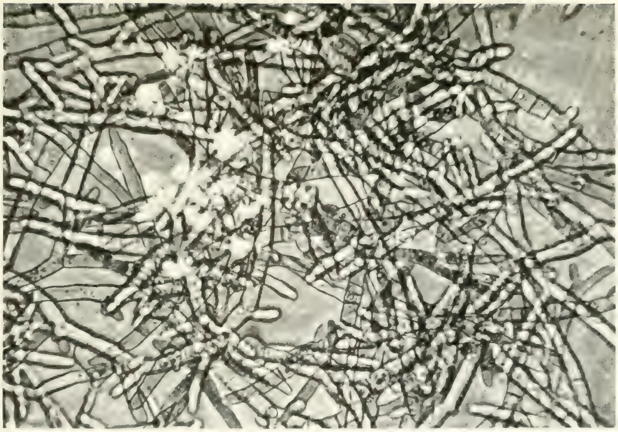
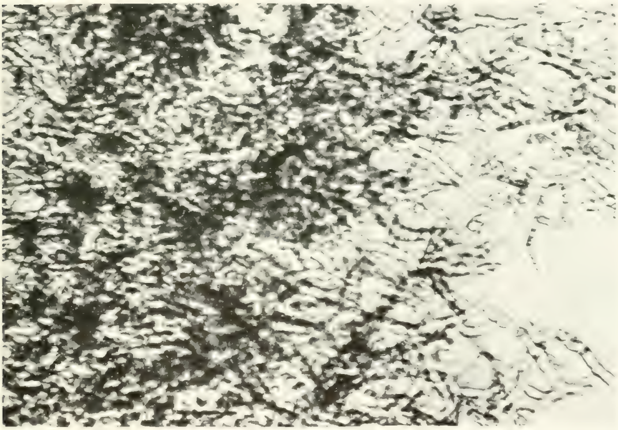


PLATE XXV

Creosote.

1. Penicillium, mycelium from 0.04 per cent solution, showing short branches and increased number of septa. The colony was firm and difficult to separate.
2. Alternaria, mycelium from 0.02 per cent solution, showing stunting and distortion. The colony had similar characteristics to the Penicillium.
3. Oidium, hyphae from 0.05 per cent solution, showing reduction of protoplasm.





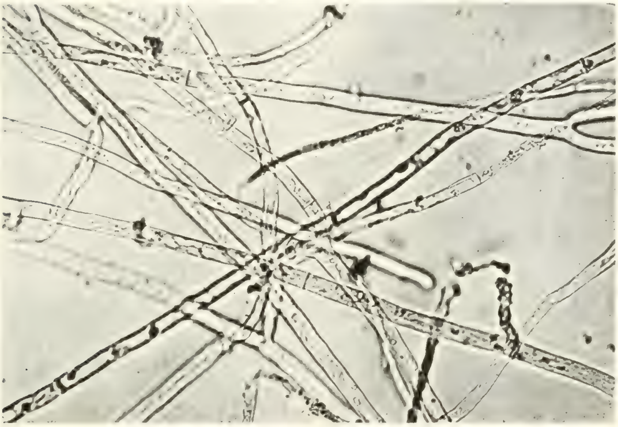


PLATE XXVI

Formaldehyde.

1. Penicillium, mycelium and fruiting heads from 0.1 per cent solution, showing stunting and increased number of septa.
2. Alternaria, mycelium from 0.02 per cent solution, showing only a slight reduction, in this the colonies developed very slowly.
3. Oidium, mycelium from 0.1 per cent solution, showing marked attenuation.

PLATE XXVII

Formaldehyde.

1. Penicillium, mycelium from 0.01 per cent solution, showing swelling.
2. Alternaria, mycelium from 0.03 per cent solution, showing some enlargement.
3. Oidium, hyphae from 0.1 per cent solution, showing swelling.

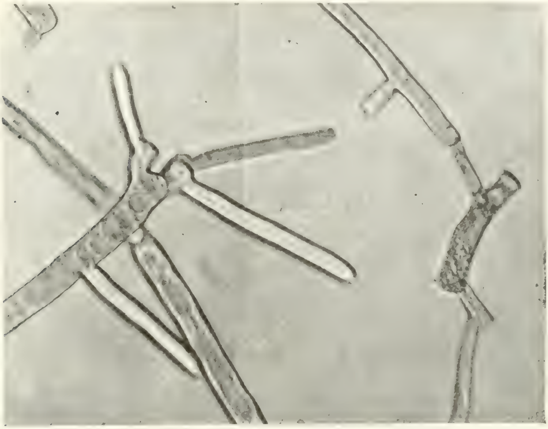
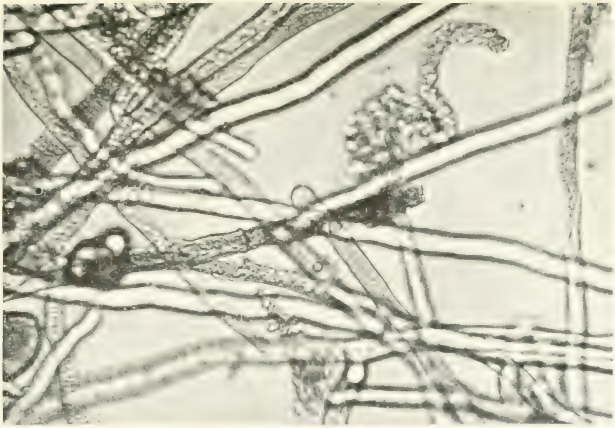


PLATE XXVIII

Formic acid.

1. Penicillium, mycelium from 0.2 per cent solution, showing marked disorganization and swelling.
2. Alternaria, mycelium from 0.5 per cent solution showing disorganization of the plasma.
3. Oidium, mycelium from 0.2 per cent solution, showing disorganization.



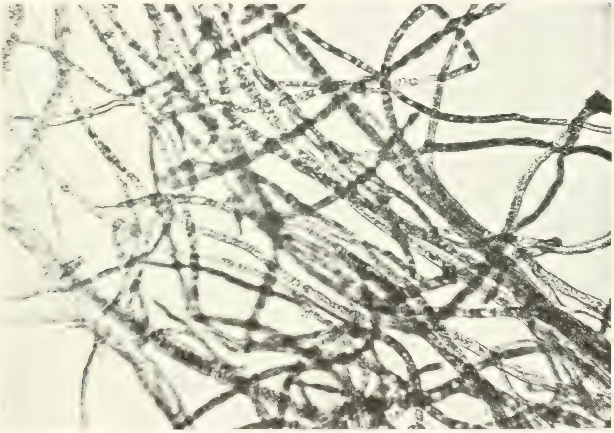
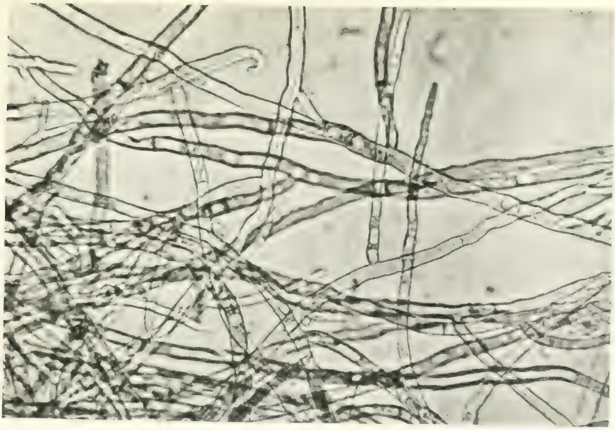
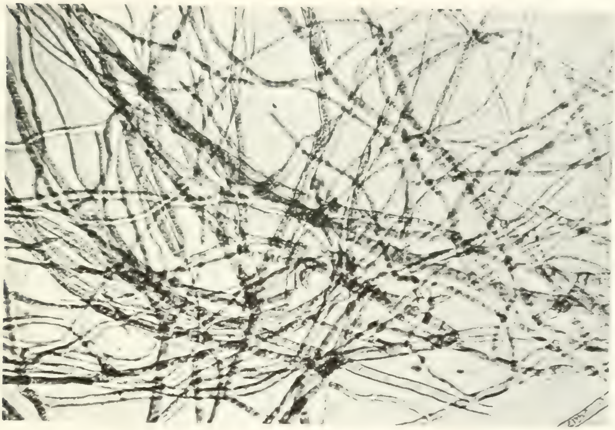


PLATE XXIX

Sodium formate.

1. Penicillium, mycelium grown in 0.2 per cent solution, showing disorganization, tips specially weak.
2. Alternaria, mycelium from 0.5 per cent solution, showing disorganization.
3. Oidium, mycelium from 0.5 per cent solution, showing disorganization.





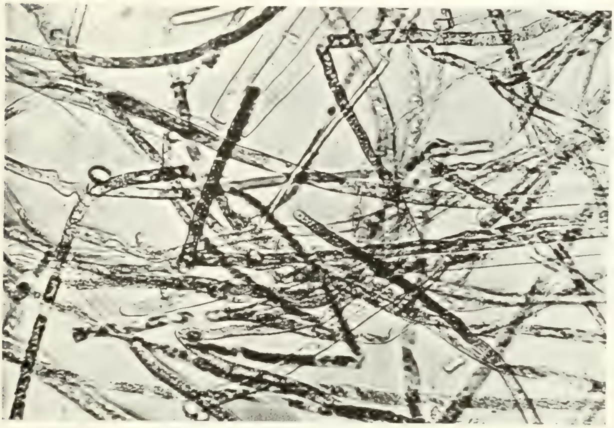
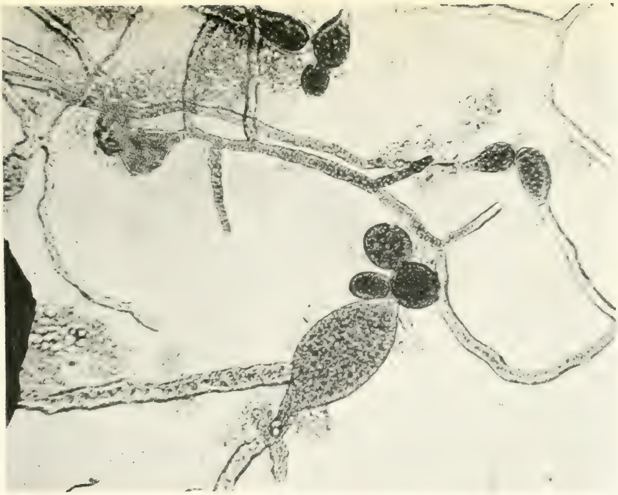
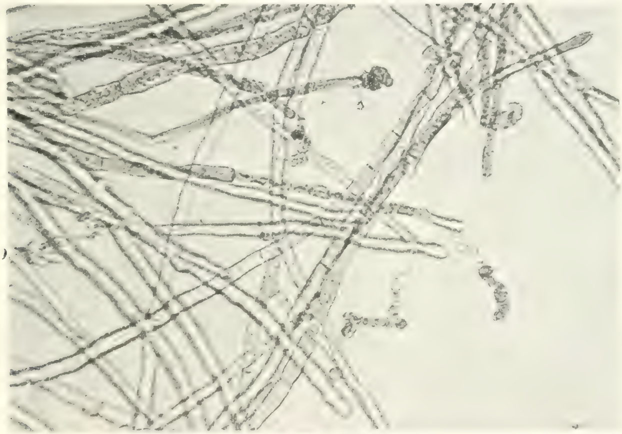


PLATE XXX

Salicylic acid.

1. Penicillium, hyphae and conidia from 0.2 per cent solution, showing excessive swelling and disorganization.
2. Alternaria, mycelium from 0.02 per cent solution, showing disorganization, tips ruptured.
3. Oidium, hyphae from 0.05 per cent solution, showing some swelling and disorganization.





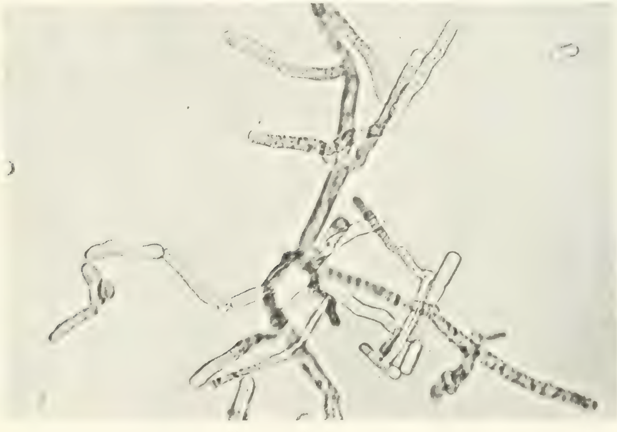


PLATE XXXI

Sodium salicylate.

1. Penicillium, mycelium from 0.2 per cent solution, showing swelling and marked disorganization. The protoplasm in the dark hyphae was brown.
2. Alternaria, mycelium from 0.5 per cent solution, showing disorganization.
3. Oidium, mycelium from 0.05 per cent solution, showing disorganization. The normal appearing oidia are from the surface.

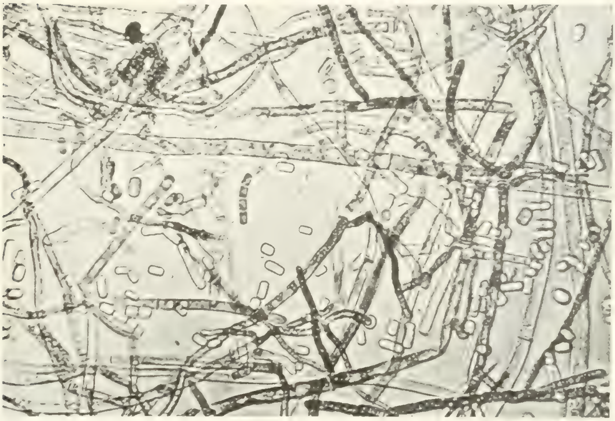


PLATE XXXII

Saccharin.

1. Penicillium, mycelium from 0.2 per cent solution, showing enlargement and disorganization.
2. Alternaria, mycelium from 0.02 per cent solution, showing excessive enlargement in parts and disorganization.
3. Oidium, mycelium from 0.3 per cent solution, showing disorganization of plasma.

PLATE XXXIII

Saccharin.

1. Penicillium, whole plant from 1 per cent solution, showing stunting.
2. Alternaria, showing disorganization of protoplasm and walls.

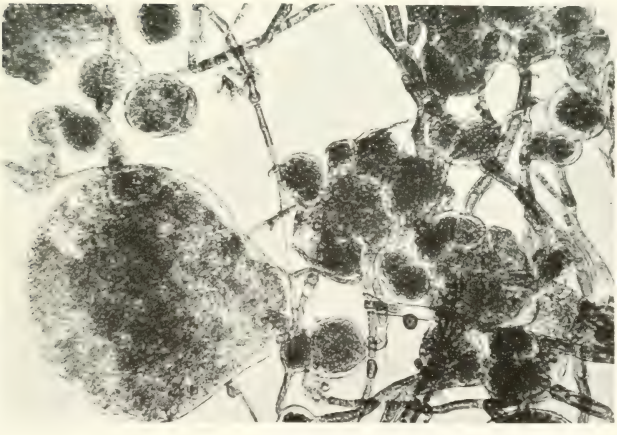
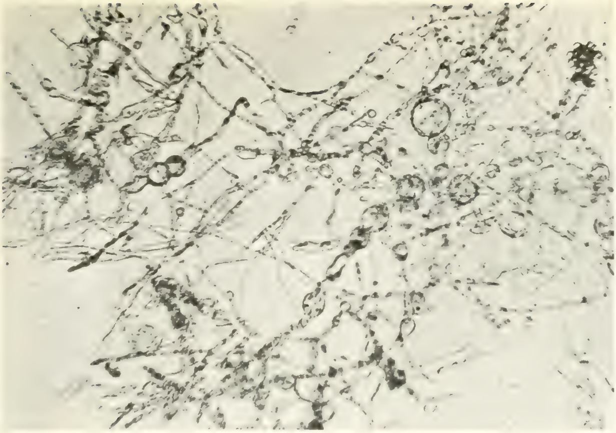
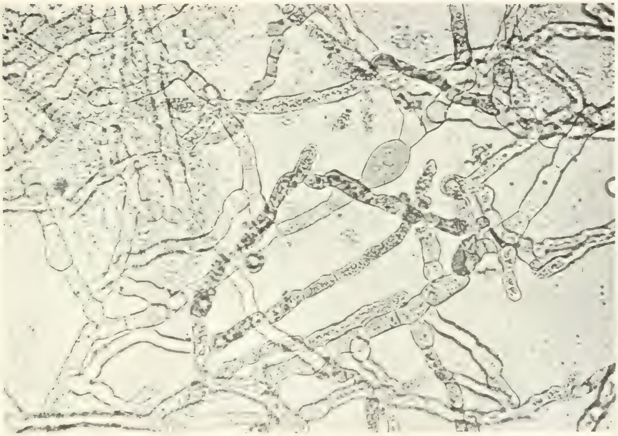


PLATE XXXIV

Alum.

1. Penicillium, mycelium from 1 per cent solution, showing decided stunting of hyphae.
2. Alternaria, mycelium from 0.5 per cent solution, showing weakening of hyphae.
3. Oidium, mycelium from 1 per cent solution, showing weakening of hyphae.





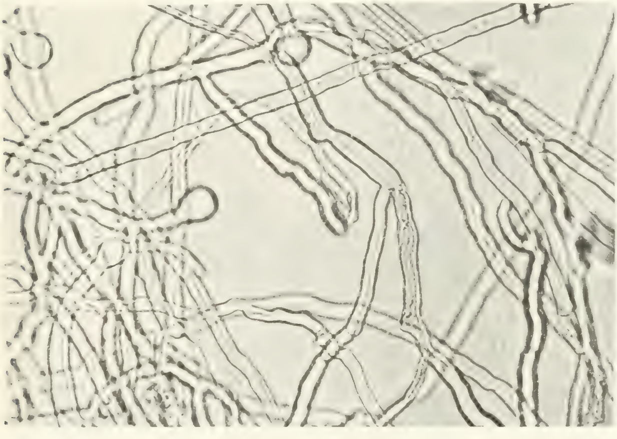
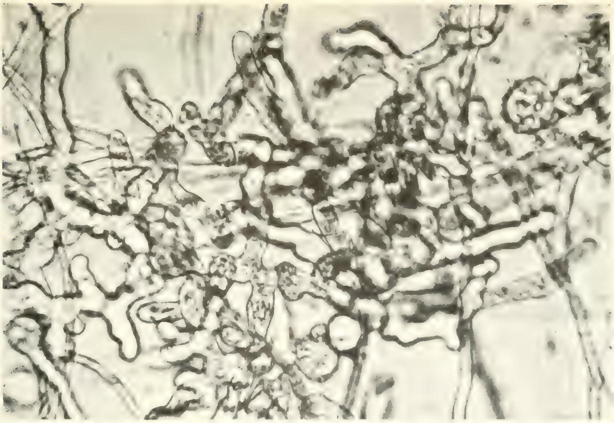


PLATE XXXV

Copper sulphate.

1. Penicillium, mycelium from 0.5 per cent solution, showing swelling, distortion, and disorganization.
2. Alternaria, mycelium from 0.1 per cent solution, showing enlargement and disorganization. In some mounts the tips ruptured.
3. Oidium, mycelium from 0.5 per cent solution, showing swelling, distortion, and disorganization.



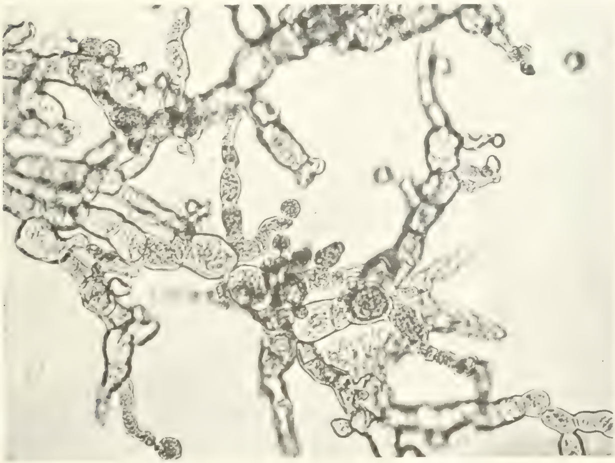


PLATE XXXVI

Copper sulphate.

1. Penicillium, hyphae from 1 per cent solution, showing marked stunting.
2. Oidium, hyphae and oidia, from 0.3 per cent solution, showing coagulation of protoplasm and coarse granules.

8

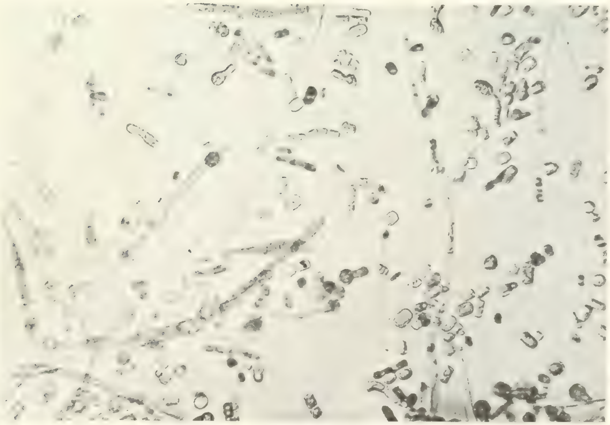


PLATE XXXVII

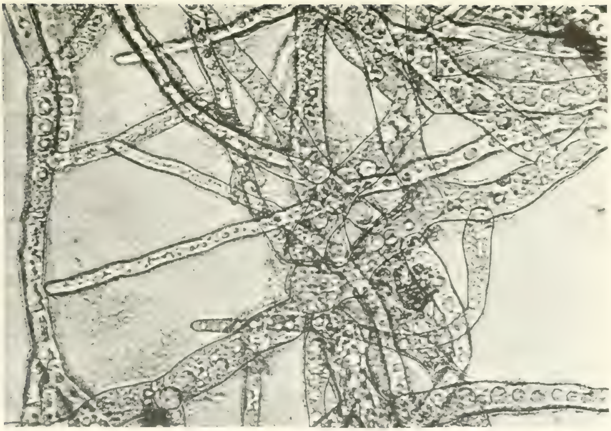
Ferrous sulphate.

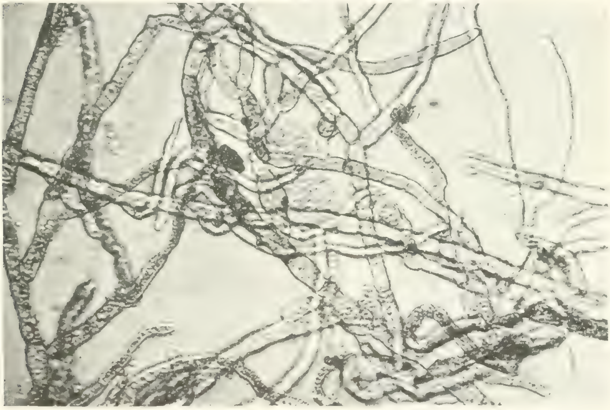
1. Penicillium, mycelium from 0.05 solution, showing some enlargement and coarse plasma.
2. Alternaria, mycelium from 0.2 per cent solution, showing enlargement of some parts, and coarse plasma.
3. Oidium, mycelium from 1 per cent solution, showing swelling and disorganization.

PLATE XXXVIII

Lead acetate.

1. Penicillium, mycelium from 0.05 per cent solution, showing swelling and disorganization.
2. Alternaria, mycelium from 0.3 per cent solution, showing enlargement and plasma coarsely granular.
3. Oidium, hyphae and oidia from 0.5 per cent solution. Hyphae have weakened appearance.





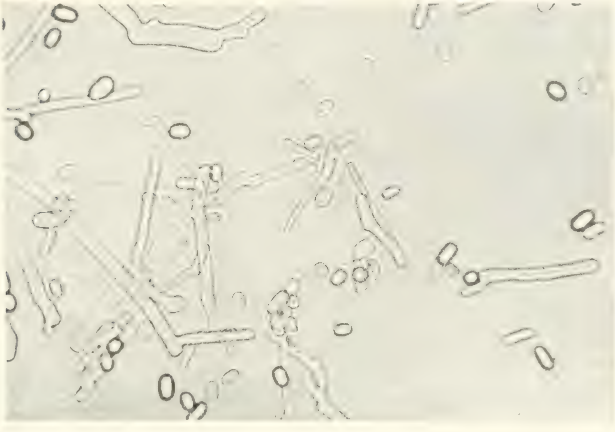
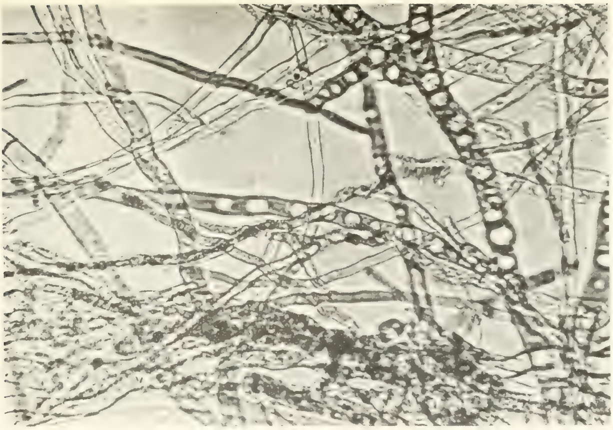


PLATE XXXIX

Sodium sulphate.

1. Penicillium, mycelium from 2 per cent solution, showing swelling and disorganization.
2. Alternaria, mycelium from 1 per cent solution, showing disorganization.
3. Oidium, mycelium from 2 per cent solution, showing weakness and disorganization. One hypha has an intergrowth with oidia formed.



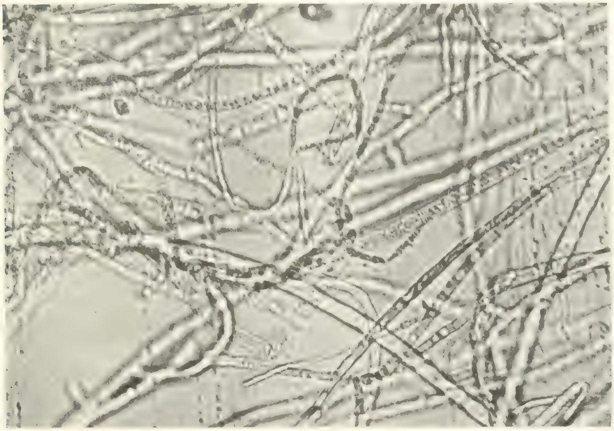


PLATE XL

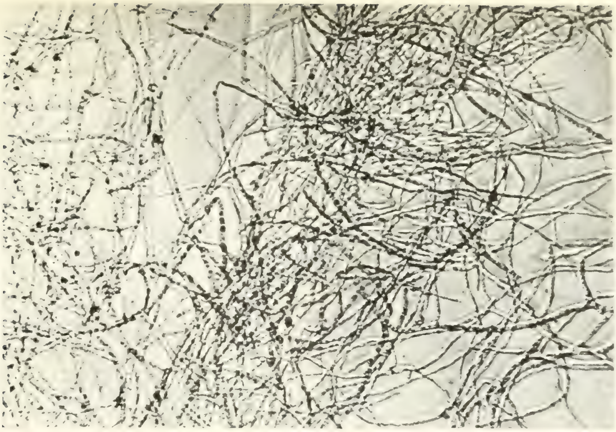
Sodium sulphite.

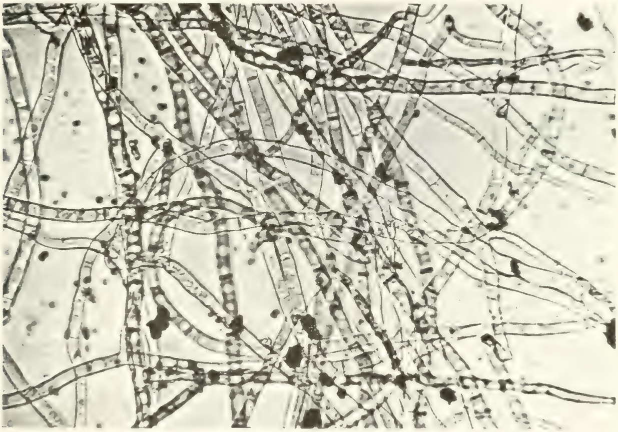
1. Penicillium, mycelium from 0.2 per cent solution, showing swelling and disorganization.
2. Alternaria, mycelium from 0.2 per cent solution, showing disorganization, tip ruptured.
3. Oidium, hyphae from 3 per cent solution, have weakened appearance.

PLATE XLI

Stannous oxide.

1. Penicillium, mycelium from 5 per cent solution, showing stunting and coagulation of plasma.
2. Alternaria, mycelium from 5 per cent solution, normal appearance. The black spots are the particles of the tin compound.
3. Oidium, mycelium from 5 per cent solution, plasma coarsened.





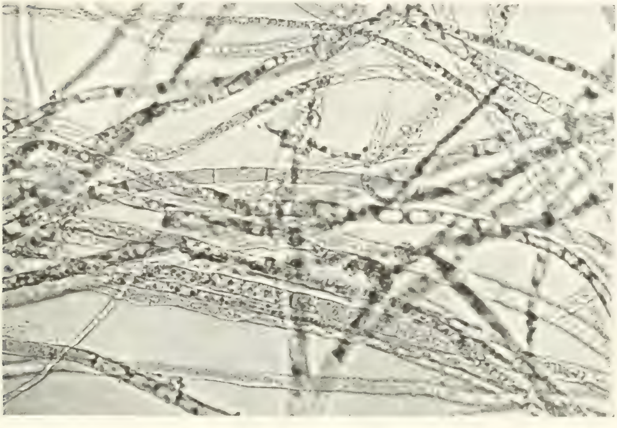


PLATE XLII

Stannous phosphate.

1. Penicillium, mycelium from 2 per cent solution, showing swelling and disorganization. Walls rupture, plasma flows out.
2. Alternaria, mycelium from 1 per cent solution, showing attenuation of hyphae.
3. Oidium, mycelium from 3 per cent solution, showing disorganization.

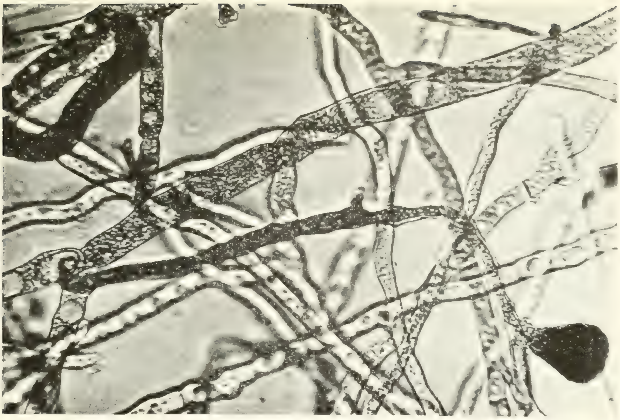


PLATE XLIII

Stannous protochloride.

1. Penicillium, mycelium from 0.02 per cent solution, showing enlargement and some disorganization evidenced in coarsened plasma.
2. Alternaria, mycelium from 0.1 per cent solution, showing disorganization in some hyphae.
3. Oidium, mycelium and oidia from 0.5 per cent solution, showing disorganization.

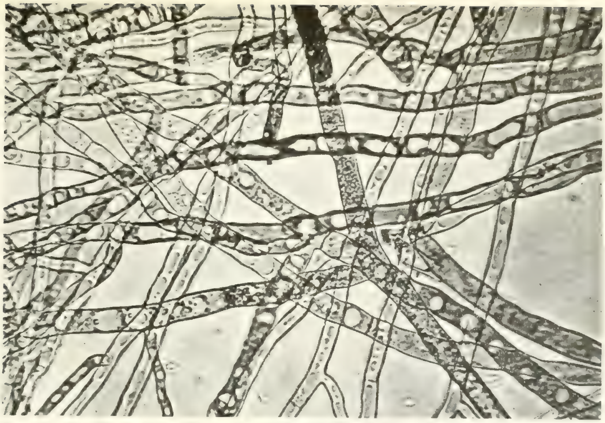


PLATE XLIV

Stannous sulphate.

1. Penicillium, mycelium from 0.02 per cent solution, showing enlargement.
2. Alternaria, mycelium from 0.1 per cent solution, showing some enlargement, weakening, and disorganization.
3. Oidium, mycelium from 0.05 per cent solution, showing some disorganization, seen in the hyphae having structureless plasma.

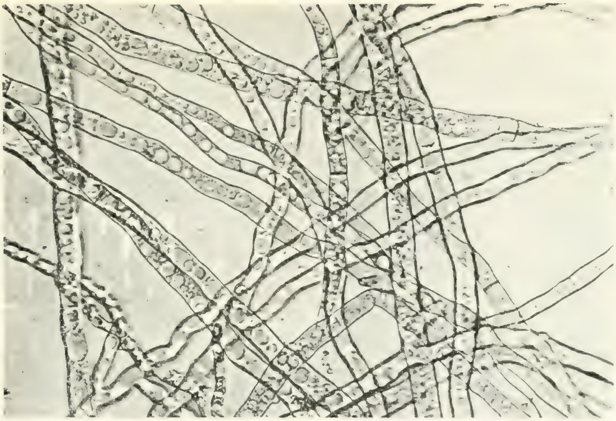


PLATE XLV

Zinc chloride.

1. Penicillium, mycelium from 0.2 per cent solution, showing swelling and disorganization.
2. Alternaria, mycelium from 0.2 per cent solution, showing swelling and disorganization, some hyphal tips ruptured.
3. Oidium, mycelium from 0.5 per cent solution, showing swelling and disorganization.

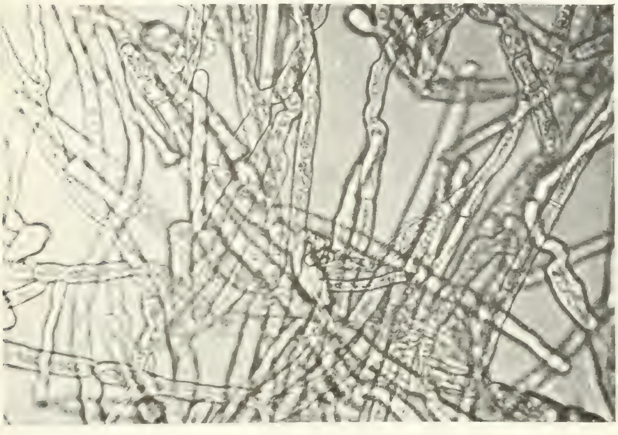
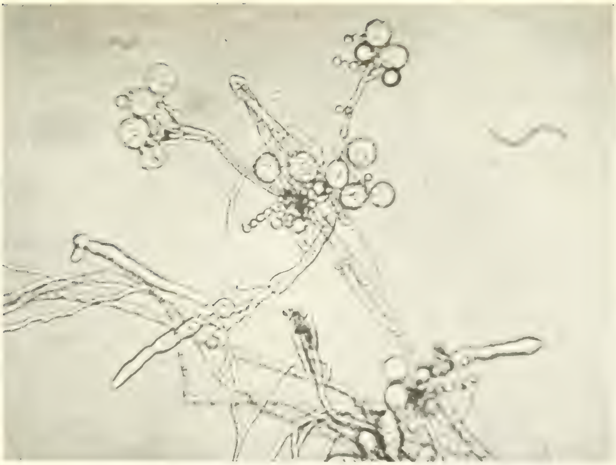
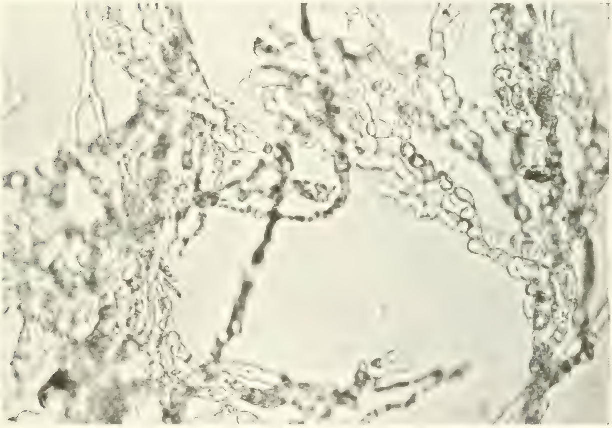


PLATE XLVI

Hydrochloric acid.

1. Penicillium, mycelium from 0.5 per cent solution, showing stunting and distortion; note the variation in size of conidia in chain on fruiting heads.
2. Alternaria, mycelium from 0.5 per cent solution, showing stunting and distortion.
3. Oidium, mycelium from 0.5 per cent solution, showing stunting and distortion.





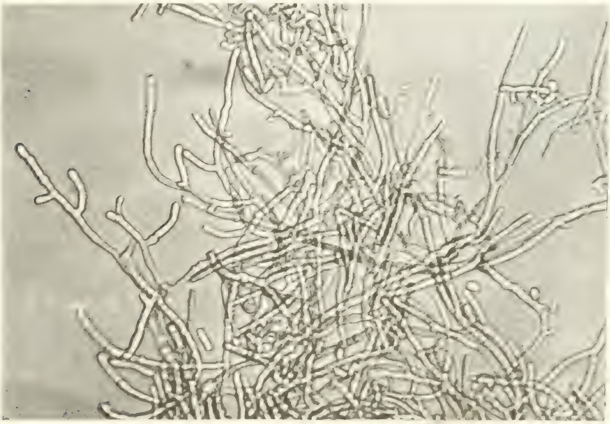
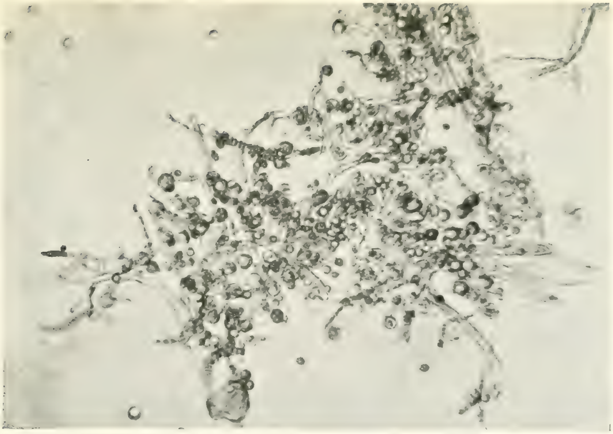
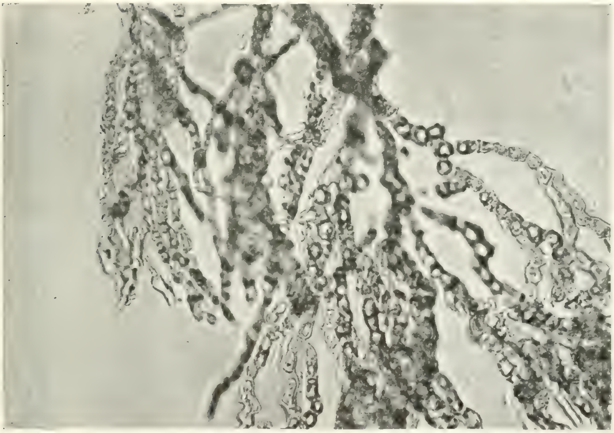


PLATE XLVII

Nitric acid.

1. Penicillium, mycelium from 0.5 per cent solution, showing stunting and distortion.
2. Alternaria, mycelium from 0.2 per cent solution, showing stunting and distortion.
3. Oidium, mycelium from 0.2 per cent solution, showing disorganization in some hyphae.





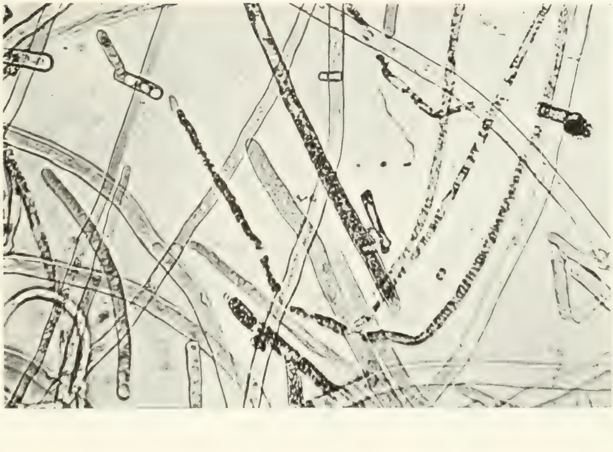
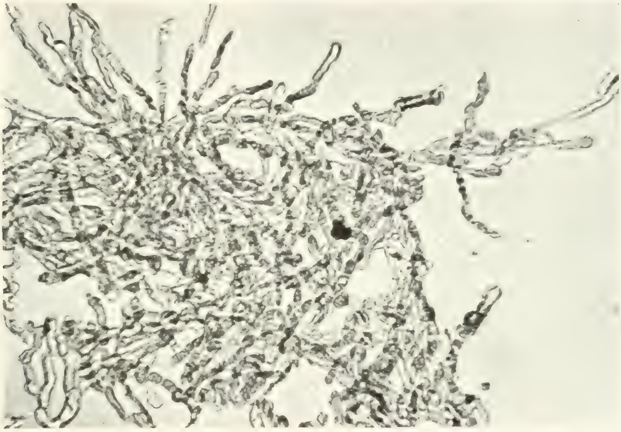


PLATE XLVIII

Sulphuric acid.

1. Penicillium, mycelium from 0.1 per cent solution. The hyphae are weak-looking and hazy with the plasma disorganized.
2. Alternaria, mycelium from 0.1 per cent solution, showing stunting and distortion.
3. Oidium, mycelium from 0.1 per cent solution, showing distortion.



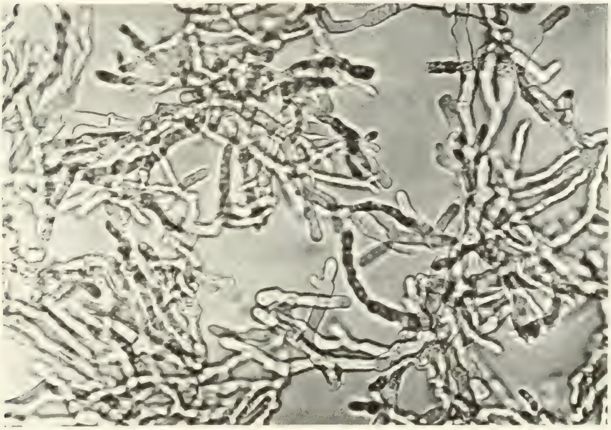
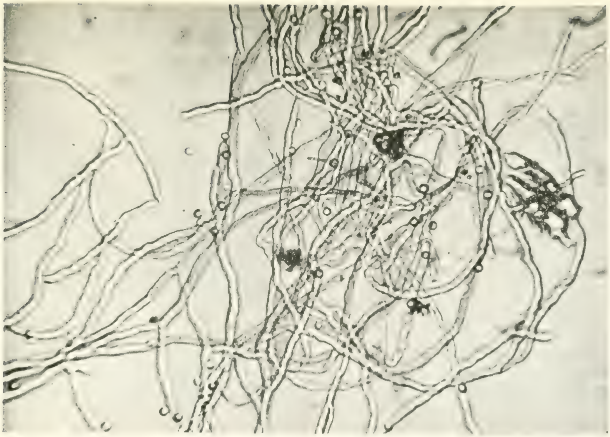
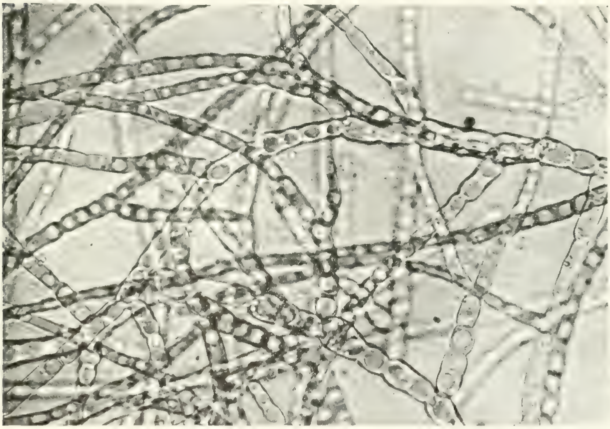


PLATE XLIX

Potassium hydrate.

1. Penicillium, mycelium from 0.5 per cent solution, showing stunting.
2. Alternaria, mycelium from 0.5 per cent solution, growth hastened, evidenced by large size and number of vacuoles.
3. Oidium, hyphae from 0.5 per cent solution, have a weak appearance.





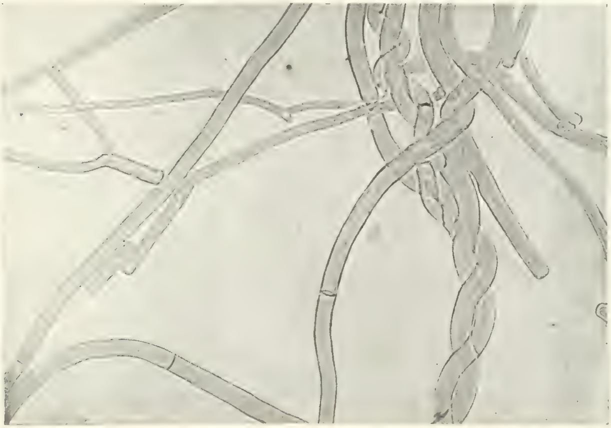
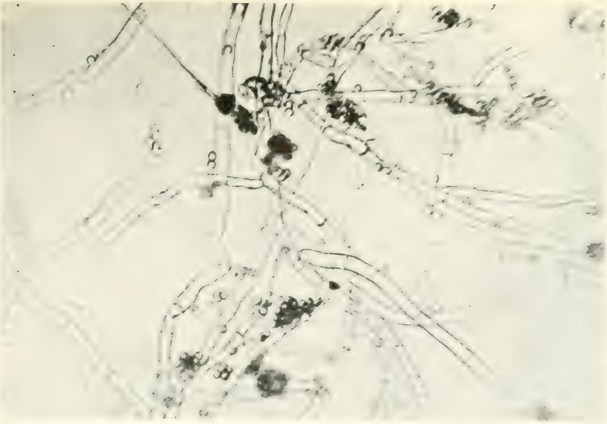
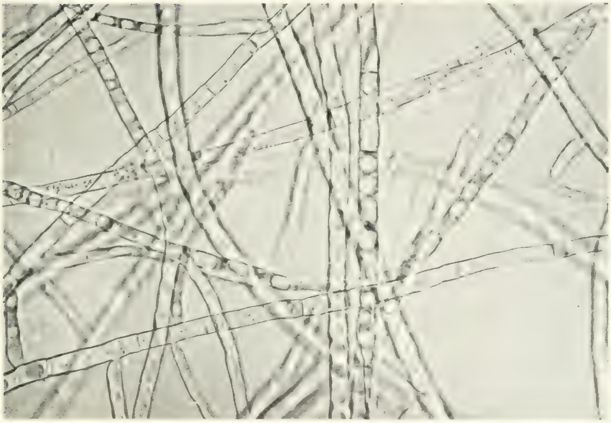


PLATE L

Sodium hydrate.

1. Penicillium, mycelium from 0.5 per cent solution, showing hazy, weak appearance.
2. Alternaria, mycelium from 0.2 per cent solution, showing hastened growth, but weak appearance.
3. Oidium, mycelium from 0.5 per cent solution, showing hyphae abnormally transparent.





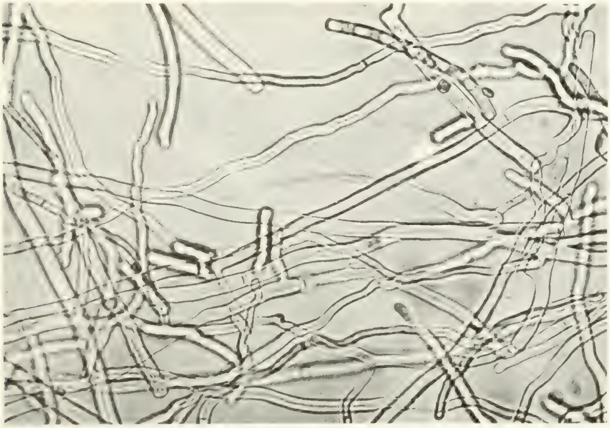


PLATE LI

Alkaloids.

1. Penicillium, mycelium from 0.0065 per cent solution atropin sulphate, showing stunting and distortion.
2. Penicillium, mycelium and fructification from 0.1625 per cent solution cocain hydrochlorate, showing stunting and distortion.
3. Penicillium, mycelium from 0.1625 per cent solution morphin sulphate, showing stunting and distortion.

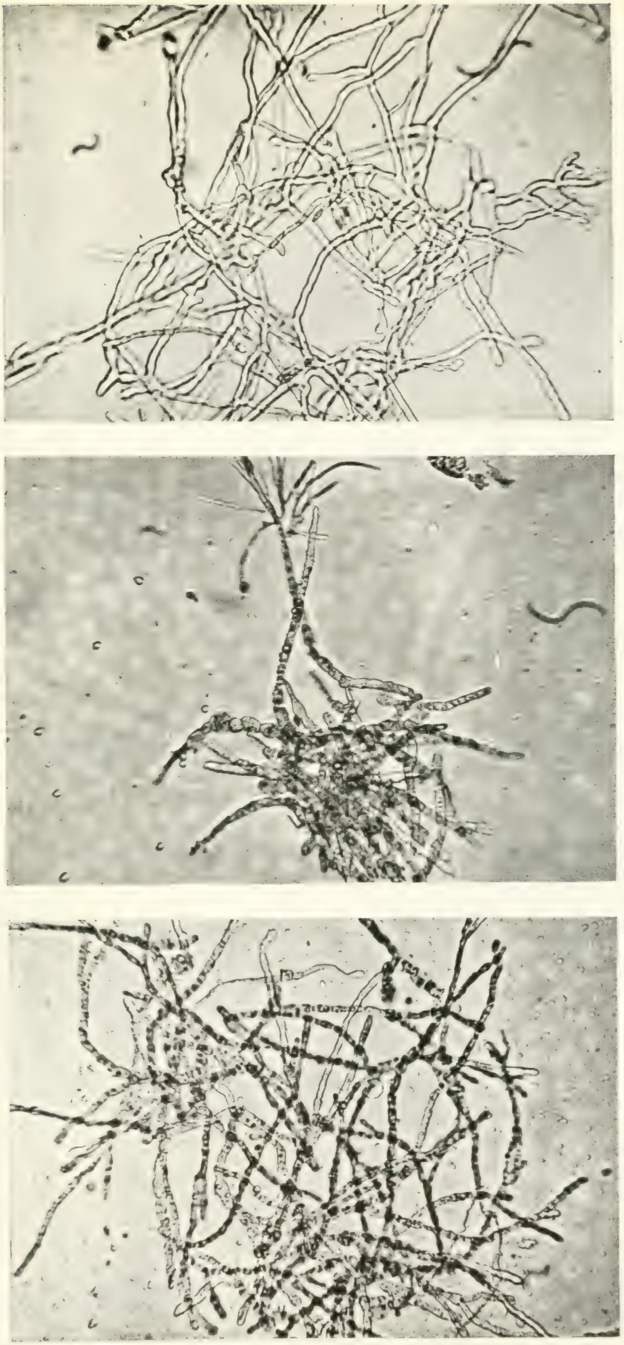


PLATE LII

Alkaloids.

1. Penicillium, mycelium from 0.0065 per cent solution strychnin nitrate, showing stunting.
2. Penicillium, mycelium from 0.01 per cent solution quinin sulphate, showing stunting and distortion.

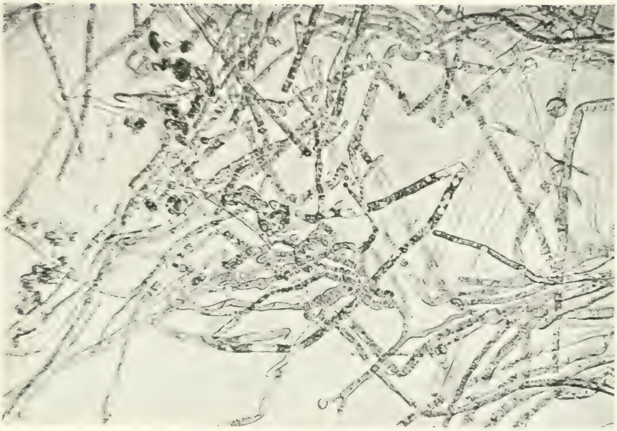
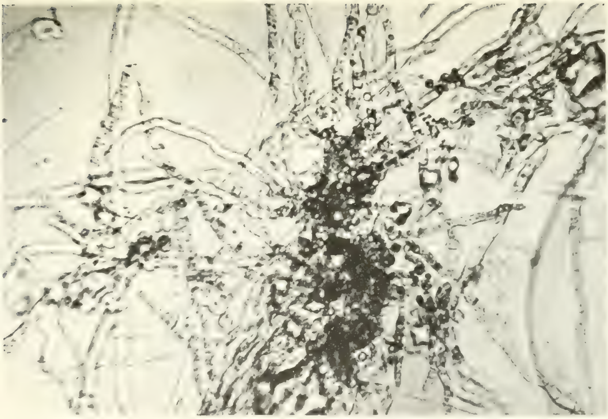


PLATE LIII

Mercuric chloride.

1. Penicillium, mycelium from 0.01 per cent solution, showing disorganization of plasma.
2. Alternaria, showing hyphae with reduced plasma, from 0.01 per cent solution.
3. Oidium, mycelium from 0.01 per cent solution, showing disorganization of plasma.



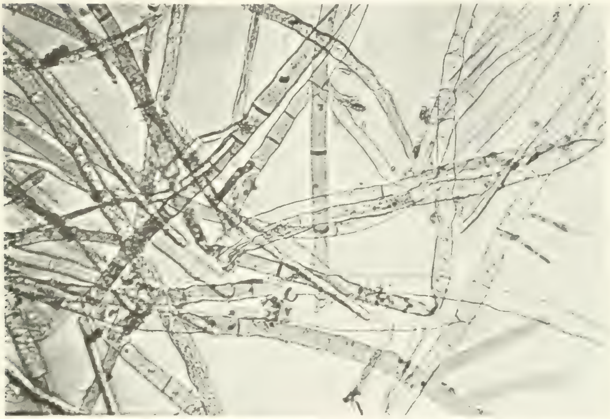


PLATE LIV

Carbolic acid.

1. Penicillium, mycelium from 0.1 per cent solution, showing swelling, specially of germinated conidia, increased number of septa, and disorganization of the plasma.
2. Alternaria, mycelium from 0.02 per cent solution, showing strong disorganization.
3. Oidium, hyphae and oidia from 0.1 per cent solution, showing swelling in some oidia.

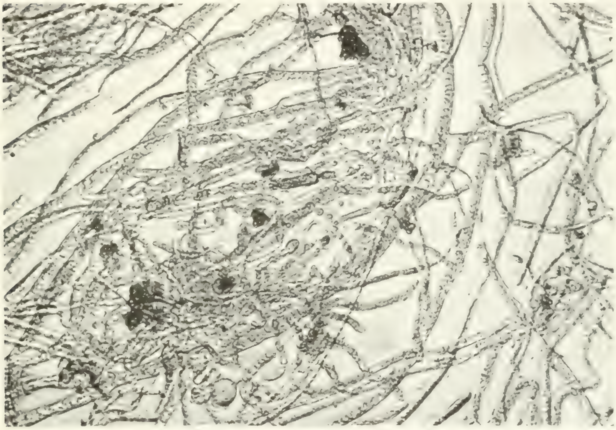
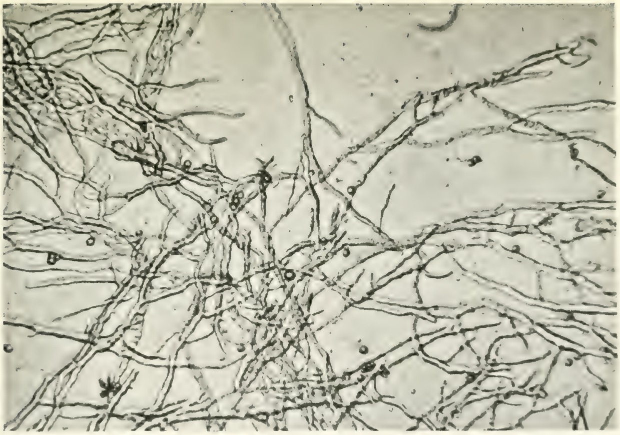


PLATE LV

Oxalic acid.

1. Penicillium, mycelium from 1 per cent solution, showing stunting and distortion.
2. Alternaria, mycelium from 0.5 per cent solution, showing decided stunting.
3. Oidium, hyphae from 0.5 per cent solution, showing enlargement and disorganization.



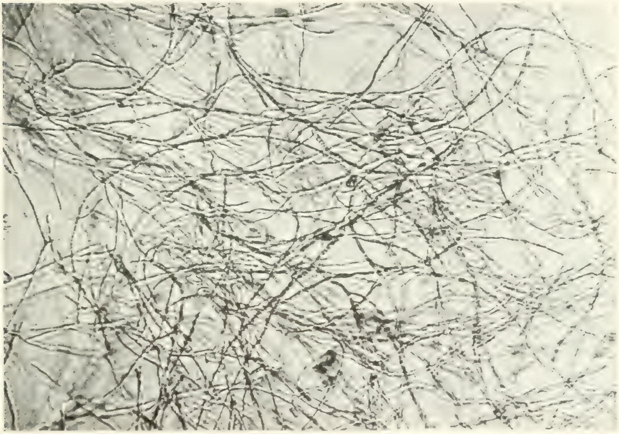
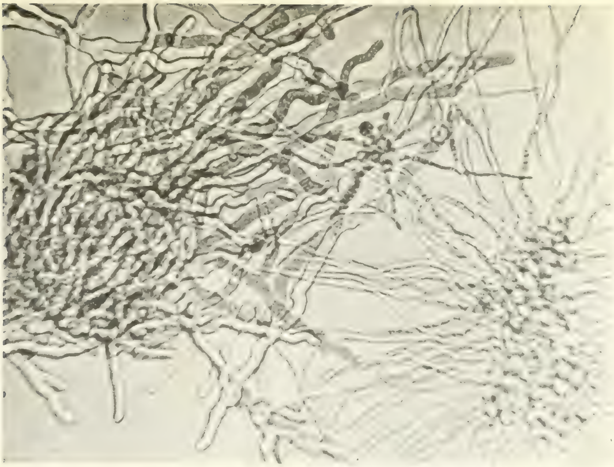


PLATE LVI

Mixtures.

1. Mycelia from solution containing 7.5 per cent salt and 0.75 per cent acetic acid. The effect of both agents is shown in the two mycelia, the one showing stunting, the other swelling.
2. Mycelia from solution containing 32.5 per cent sugar and 0.75 per cent acetic acid. In this the extreme effect of the acetic acid is seen, but no effect of the sugar.
3. Mycelia from solution containing 32.5 per cent sugar and 0.1 per cent sodium benzoate. The hyphae show a selective action, some the attenuation due to the sugar, others the swelling and disorganization due to the sodium benzoate.



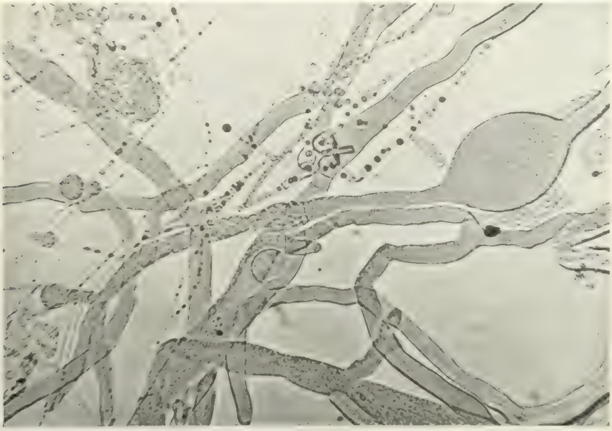


PLATE LVII

Mixtures.

1. Conidia from solution containing 0.75 per cent acetic acid and 0.1 per cent sodium benzoate. The result appears as a combined action, both agents causing swelling and disorganization.
2. Hyphae and conidia from solution containing 16.25 per cent sugar, 3.75 per cent salt, 0.375 per cent acetic acid, and 0.05 per cent sodium benzoate. Only the action of the acetic acid and sodium benzoate is seen in the swelling and disorganization.
3. Hyphae and conidia from solution containing 3.75 per cent salt, 0.375 per cent acetic acid, and .05 per cent sodium benzoate. In this culture the action of the three agents is noted in the stunting of some and the swelling and disorganization of others.

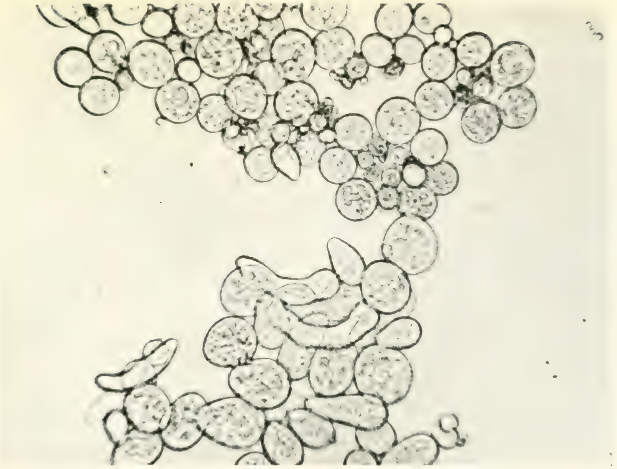


PLATE LVIII

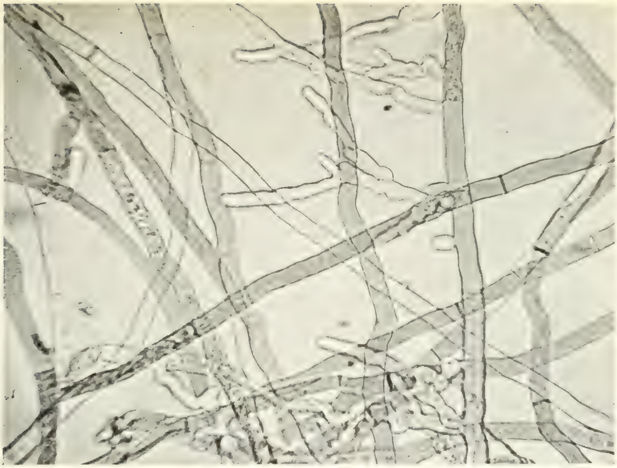
Penicillium, the length of time required for germination is given in the table on results of germination tests.

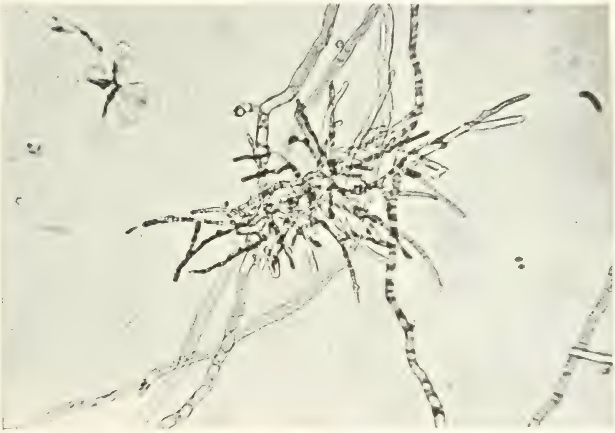
1. Control, young.
2. Control, 590 days, showing hazy appearance and somewhat stunted fruiting heads.
3. Salt solution, 15 per cent. The mycelium shows decided stunting, the effect persisting.

PLATE LIX

Penicillium.

1. Cinnamon, 10 per cent solution, 590 days. The mycelium shows enlargement.
2. Distilled vinegar, 1 per cent, 380 days. The hyphae show stunting.
3. Lactic acid, 10 per cent solution. The hyphae show disorganization.





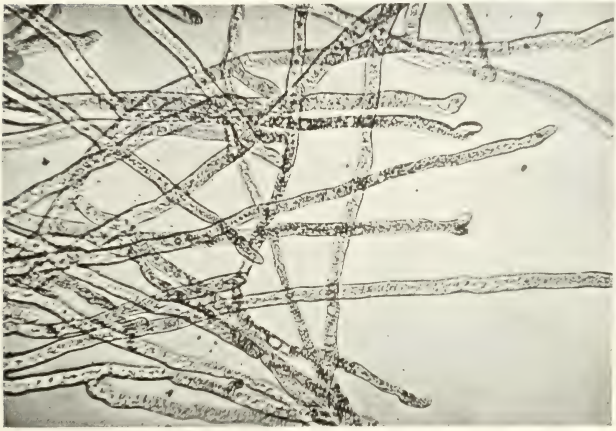
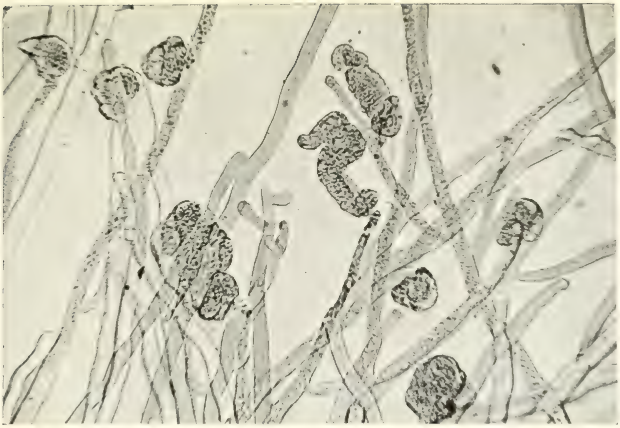


PLATE LX

Penicillium.

1. Butyric acid, 0.1 per cent, 133 days. The hyphae show swelling and disorganization.
2. Borax, 0.2 per cent, 423 days. The effect was mainly stunting.
3. Sodium salicylate, 0.2 per cent, 590 days. Swelling and disorganization shown.



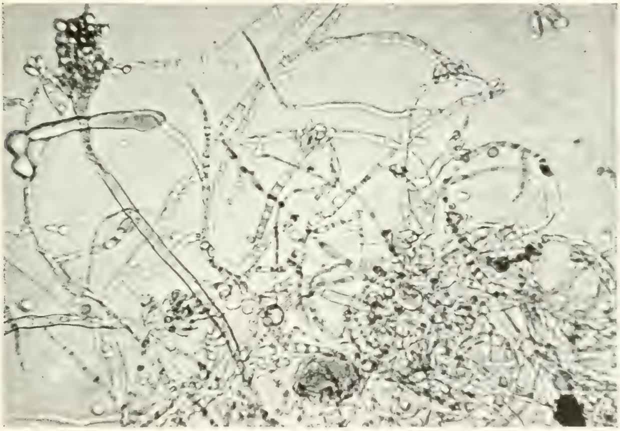
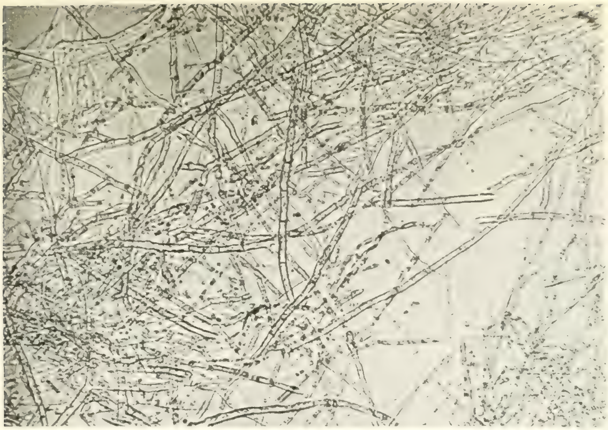


PLATE LXI

Penicillium.

1. Sodium sulphite, 0.2 per cent, 590 days. The hyphae show decided stunting.
2. Sodium sulphite, 0.5 per cent, 590 days. The hyphae here show swelling and disorganization of plasma.
3. Copper sulphate, 0.2 per cent, 590 days. The hyphae show decided stunting.



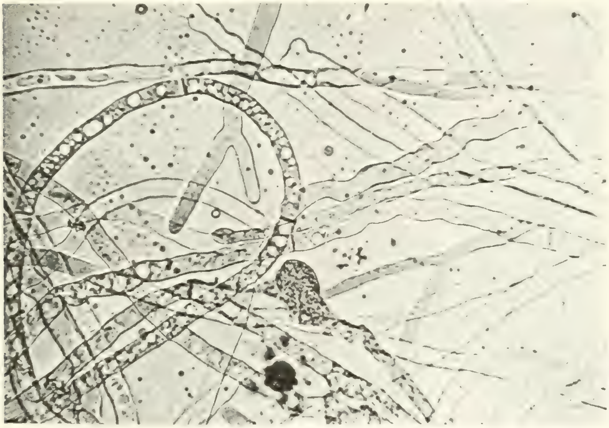
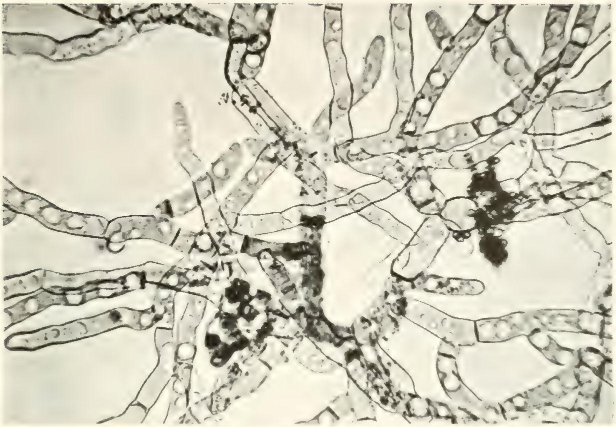


PLATE LXII

Penicillium.

1. Zinc chloride, 0.1 per cent, 133 days. The hyphae are swollen.
2. Nitric acid, 0.5 per cent, 30 days. Some fruiting heads stunted, others abnormally bushy.



TX
607
B57

Bitting, Katherine (Golden)
The effect of certain agents on the development of some moulds

BioMed

PLEASE DO NOT REMOVE
CARDS OR SLIPS FROM THIS POCKET

UNIVERSITY OF TORONTO LIBRARY